

## BLOOD PRESSURE CONTROL IN THE ANTARCTIC FISH *PAGOTHENIA BORCHGREVINKI*

MICHAEL AXELSSON\*, BILL DAVISON, MALCOLM FORSTER  
AND STEFAN NILSSON†

*Department of Zoology, University of Canterbury, Private Bag 4800, Christchurch,  
New Zealand*

*Accepted 31 January 1994*

### Summary

The mechanisms of cardiovascular control in the Antarctic fish *Pagothenia borchgrevinki* were investigated during rest and swimming exercise using pharmacological tools to reveal the nature of the control systems involved. Simultaneous and continuous recordings of ventral and dorsal aortic blood pressure, heart rate and ventral aortic blood flow (cardiac output) were made using standard cannulation procedures and a single-crystal Doppler flowmeter.

Exercise produced a clear and consistent decrease in dorsal aortic blood pressure caused by a decrease in systemic vascular resistance. At the same time, ventral aortic blood pressure increased owing to the combined effects of a markedly increased cardiac output (by about 80%) and branchial vasoconstriction. Judged from the effects of the  $\alpha$ -adrenoceptor antagonist prazosin, control of the branchial vasculature involves an  $\alpha$ -adrenoceptor-mediated vasoconstriction, in addition to more traditional cholinergic vasoconstrictor and  $\beta$ -adrenoceptor-mediated dilatory mechanisms.

The range of heart rates is large, from 3–4 beats  $\text{min}^{-1}$  in individual fish during hypertensive bradycardia to about 28 beats  $\text{min}^{-1}$  after atropine treatment. Both chronotropic and inotropic effects are responsible for a marked increase in cardiac output during exercise. The increase in blood pressure caused by adrenaline injection was due largely to an increase in cardiac output, while direct effects on the systemic vasculature were small and transient. The increase in cardiac output, in turn, was due solely to an adrenergic stimulation of stroke volume. A barostatic bradycardia, often seen in other vertebrates in response to adrenaline injection, was absent and it is possible that a decrease in heart rate was offset by direct adrenergic stimulation of the heart.

Angiotensin II (Ang II) produced consistent hypertension by systemic vasoconstriction. In contrast to the effects of adrenaline injection, the hypertension caused by Ang II was accompanied by a marked bradycardia. This could be abolished by atropine, suggesting a cholinergic vagal reflex of the type found in other vertebrates.

\*Present address: Comparative Neuroscience Unit, Department of Zoophysiology, University of Göteborg, Medicinargatan 18, S-413 90 Göteborg, Sweden.

†Present address and address for reprint requests: Department of Zoophysiology, University of Göteborg, Medicinargatan 18, S-413 90 Göteborg, Sweden.

Key words: *Pagothenia borchgrevinki*, blood pressure, exercise, vascular control, catecholamines, angiotensin.

Angiotensin I also caused an elevated blood pressure, and this effect was abolished by the angiotensin converting enzyme inhibitor enalapril, demonstrating elements of an angiotensin-related cardiovascular control system.

### Introduction

Antarctic fish of the sub-Order Notothenioidei live at constant low temperatures south of the Antarctic convergence. This group of fish includes both the haemoglobin-free ice fish (family Channichthyidae) and the red-blooded nototheniids (family Nototheniidae). Notothenioids maintain a high cardiac output despite a low heart rate. In a previous preliminary study, the cryopelagic nototheniid *Pagothenia borchgrevinki* (Boulenger) showed a heart rate of 11 beats  $\text{min}^{-1}$  while maintaining a cardiac output estimated to be about 30  $\text{ml min}^{-1} \text{kg}^{-1}$ . Total vascular resistance was low (Axelsson *et al.* 1992). *Pagothenia borchgrevinki* has a low resting haematocrit, which increases substantially when demand for increased oxygen transport is induced by various types of stimuli, including handling, exercise and hypoxia (Macdonald *et al.* 1987; Davison *et al.* 1988; Franklin *et al.* 1993). The low resting haematocrit contributes to the low vascular resistance as it reduces blood viscosity, a major factor at low temperatures (Farrell, 1984; Macdonald and Wells, 1991; Axelsson *et al.* 1992).

The low heart rate depended largely on a tonic vagal cholinergic influence, with atropine producing a doubling of the heart rate. Atropine also produces a marked decrease in ventral aortic blood pressure (Axelsson *et al.* 1992), which could be interpreted as indicating a tonic cholinergic vasoconstrictor influence on the branchial vascular resistance (see Smith, 1977; Pettersson and Nilsson, 1979). However, as the study of Axelsson *et al.* (1992) did not measure dorsal aortic blood pressure, separate effects on the gill vasculature and systemic vasculature could not be determined.

The role of the sympathico-adrenal system in the regulation of blood pressure in vertebrates has received considerable attention. In teleost fish, circulating catecholamines, released from both chromaffin tissue and the adrenergic neurones of the autonomic nervous system, have been implicated as the cause of an adrenergic tonus, affecting the heart rate and vascular resistance both at rest and during exercise (e.g. Randall and Stevens, 1967; Cameron, 1979; Smith *et al.* 1985; Axelsson and Nilsson, 1986). In addition, there is now evidence for an involvement of several other groups of substances in cardiovascular control in fish, including adenosine derivatives, serotonin (5-hydroxytryptamine), numerous neuropeptides and the renin-angiotensin system (Nilsson and Holmgren, 1992; Olson, 1992). Although we have evidence of a high inhibitory cholinergic tonus and weak excitatory adrenergic tonus on the heart of two species (Axelsson *et al.* 1992), our knowledge of cardiovascular control in Antarctic fish is very limited (Montgomery and Wells, 1993). Nothing is known of the effects of other potential control substances.

Most Antarctic fish are benthic and will not swim unless provoked, making them unsuitable for looking at methods of cardiovascular control during exercise (Egginton *et al.* 1991; Axelsson *et al.* 1992). *Pagothenia borchgrevinki* is a pelagic labriform swimmer, using its large pectoral fins for propulsion and the tail as a rudder. It will swim

readily in a swimming tunnel (Forster *et al.* 1987; Davison *et al.* 1988), making it ideal for cardiovascular studies. The present study is an investigation of patterns of blood pressure regulation in *P. borchgrevinki* at rest and during non-exhaustive swimming.

### Materials and methods

The cryopelagic red-blooded Antarctic nototheniid fish *Pagothenia borchgrevinki* (Boulenger) (commonly called 'borch') was caught by hook and line through holes cut in the fast ice covering McMurdo Sound, Antarctica. Fish were transferred to Scott Base (New Zealand Antarctic Programme) and kept in plastic tanks at  $-1$  to  $+1$  °C. They were subsequently flown to Christchurch, New Zealand, where this study was conducted. Fish were maintained in a closed-circuit aquarium system at  $-0.5$  °C until needed.

### Surgery

Fish of either sex (body mass 82.5–198 g, body length 21.5–27 cm;  $N=24$ ) were anaesthetized in sea water containing benzocaine,  $100\text{ mg l}^{-1}$ , until breathing movements stopped, and transferred to an operating table in a cold room (0 °C). A steady flow of sea water at 0 ° to  $+2$  °C containing approximately  $25\text{ mg l}^{-1}$  benzocaine was maintained over the gills throughout surgery.

Fish were placed ventral side up in an operating tray, and a ventral incision was made to expose the ventral aorta. A cuff-type single-crystal Doppler flow probe (cuff diameter 1.5 mm) was fitted around the ventral aorta, and the leads were secured by skin sutures. The fish was placed on its side, and a polyethylene cannula (PE50, pulled to provide a smaller diameter at the tip, and slightly bent to fit the curve of the artery) filled with heparinized (approximately  $100\text{ i.u. ml}^{-1}$ ) 1.4% NaCl was inserted occlusively into the efferent branchial artery of the third gill arch and pushed forward to about the level of the suprabranchial artery. This cannula was used to record dorsal aortic blood pressure ( $P_{DA}$ ) and to inject drugs. The cannula was tied to the gill arch and secured by two skin sutures. A second similar cannula (PE50 tipped with about 1 cm of PE10) was inserted into the afferent branchial artery of the same gill arch and secured by two skin sutures.

Following surgery, the fish were returned to fresh sea water to recover for at least 22 h before any experiments were conducted.

### Experimental equipment

The cannulae were attached to Honeywell model 156PC06GW2 disposable pressure transducers calibrated against a static column of water. The transducers were connected to Grass preamplifiers, and a tachograph preamplifier triggered by the pulsatile blood pressure signal was used to obtain heart rate ( $f_H$ ) data. Blood flow in the ventral aorta (cardiac output) was recorded using a Doppler flowmeter system (Iowa University). This system allows accurate measurement of blood velocity; previous studies have demonstrated a linear relationship between blood velocity and instantaneous volume flow (e.g. Axelsson *et al.* 1990; Axelsson and Fritsche, 1991; Sundin and Nilsson, 1992). However, the size of the fish and the placement of the flow probes do not allow reliable and regular posthumous calibration of volume flow, and variations in cardiac output ( $\dot{Q}$ )

are therefore retained as  $\Delta\text{kHz}$  Doppler shift and presented as percentage changes compared with initial values.

Pressure traces from the ventral and dorsal aortas ( $P_{VA}$  and  $P_{DA}$ ) and cardiac output ( $\dot{Q}$ ) were displayed and recorded using a Yokogawa model 3701 LR8100 recorder, and all data (including  $f_H$ ) were continuously sampled by a Toshiba model 5200 PC-based data-acquisition system (AD-DATA, Dr P. Thorén, Department of Physiology, Göteborg).

#### *Experimental protocol*

##### *Group I*

Individual fish ( $N=12$ ) were placed in a Blazka-type swim tunnel, which allowed a reproducible work load to be set. After recovery from surgery, each fish was exposed to a 5 min (control) swim trial at  $20\text{ cm s}^{-1}$  ( $0.8\text{--}1.0$  body lengths  $\text{s}^{-1}$ ). During recovery from this swim, once the cardiovascular variables had stabilized, a dose of adrenaline ( $5\text{ nmol kg}^{-1}$ ) was injected *via* the dorsal aortic cannula, and the effect recorded. Doses of drugs injected were based on the previous study (Axelsson *et al.* 1992). When the cardiovascular variables had again stabilized, the  $\alpha$ -adrenoceptor antagonist prazosin ( $1\text{ mg kg}^{-1}$ ) was injected and allowed to act for 1 h. When the recorded variables had stabilized, a second dose of adrenaline ( $5\text{ nmol kg}^{-1}$ ) was injected to check the integrity of the  $\alpha$ -adrenoceptor blockade. After a further 30 min, the fish was again exercised for 5 min.

To investigate a possible role of the angiotensin system in blood pressure control in *P. borchgrevinki*, angiotensin I (Ang I; from salmon;  $1\text{ nmol kg}^{-1}$ ) was injected into the fish and, when the pressor response to this drug had subsided, a dose of the angiotensin converting enzyme inhibitor enalapril ( $1\text{ mg kg}^{-1}$ ) was injected. The effects of a second dose of Ang I were then recorded, and finally the response to angiotensin II (Ang II; human;  $1\text{ nmol kg}^{-1}$ ) was recorded. The effects of the angiotensins were initially tested over the range  $0.1\text{--}100\text{ nmol g}^{-1}$ , with substantial differences in the magnitude of the response being noted in individual fish. A dose of  $1\text{ nmol g}^{-1}$  was chosen since most fish showed consistent blood pressure responses to this dose. Seven of the twelve fish in the group maintained patent cannulae and flow signals and swam for the full programme.

##### *Group II*

A second group of fish ( $N=12$ ) was subjected to a 5 min swim trial as described for group I. After recovery from this control swim period, each fish was injected with Ang II ( $1\text{ nmol g}^{-1}$ ). Although the group I fish had also been injected with Ang II, they received it after prior treatment with a number of other drugs, which might have influenced the response. In group II fish, 10 min after the injection of Ang II, atropine ( $1.0\text{ mg kg}^{-1}$ ) was injected. One hour later, a second swim trial was carried out. The  $\beta$ -adrenoceptor antagonist sotalol ( $3\text{ mg kg}^{-1}$ ) was then injected, and 1 h later the fish swam one last time. The full protocol was completed in eight of the twelve fish.

#### *Drugs*

The following drugs were used in this study: atropine base (neutralized using HCl)

(Sigma); L-adrenaline base (neutralized using HCl) (Sigma); [Asn<sup>1</sup>, Val<sup>5</sup>, Asn<sup>9</sup>]-angiotensin I, salmon, (Sigma); angiotensin II, human (Sigma); enalapril (Renitec, MSD); prazosin (Sigma) and sotalol hydrochloride (Hässle AB).

#### *Data acquisition and statistics*

Cardiovascular variables were continuously recorded using the Yokogawa recorder, and data were also sampled using the computerized data-acquisition system for later retrieval and calculations. Mean values were created at 30 s intervals from continuous sampling in 5 s blocks at 5 samples s<sup>-1</sup>, and data presented show means  $\pm$  S.E.M. for *N* animals. During swimming exercise, the tachometer-derived *f*<sub>H</sub> signal was irregular as a result of the movement of the animal. Therefore, this variable and stroke volume (*V*<sub>s</sub>), calculated from *f*<sub>H</sub>, were omitted for the swimming period in Figs 2 and 3.

Branchial and systemic vascular resistances (*R*<sub>gill</sub> and *R*<sub>sys</sub>, respectively) were calculated as  $(P_{VA} - P_{DA})/\dot{Q}$  and  $P_{DA}/\dot{Q}$ , respectively, assuming that all the cardiac output passed through the systemic vessels. Stroke volume was determined as  $\dot{Q}/f_H$ . In all cases,  $\dot{Q}$  was expressed as  $\Delta$ kHz Doppler shift, and for presentations these values have been converted to percentage changes.

Evaluation of statistically significant differences ( $P \leq 0.05$ ) in the observations was made using the Wilcoxon signed-ranks test. The sequentially rejective Bonferroni test described by Holm (1979) was used to eliminate, as far as possible, the possibility of discarding any true null hypothesis.

### **Results**

Resting values for *P*<sub>VA</sub> (3.58 $\pm$ 0.13 kPa; *N*=24) were similar to those described in the previous study (Axelsson *et al.* 1992), although mean *f*<sub>H</sub> was considerably higher at 20.6 $\pm$ 0.9 beats min<sup>-1</sup>. Resting *P*<sub>DA</sub> was 2.77 $\pm$ 0.10 kPa; *N*=24. Resting branchial vascular resistance (*R*<sub>gill</sub>) was approximately 25 % of the systemic vascular resistance (*R*<sub>sys</sub>).

Prior to treatment with drugs, all of the fish in both groups swam steadily when the current speed was increased in the swim tunnel. During exercise at 20 cm s<sup>-1</sup>, *P*<sub>DA</sub> consistently fell by 10 %, while *P*<sub>VA</sub> increased by about 18 %. Cardiac output increased dramatically by 78 %, as a result of increases in stroke volume of 36 % and in *f*<sub>H</sub> of 33 %. The fall in *P*<sub>DA</sub> was due to a decrease in *R*<sub>sys</sub>, by more than 50 %, which could not be offset by the increase in  $\dot{Q}$ . The branchial vascular resistance increased by about 30 % (Figs 1 and 2). Blood pressure and flow recovered to pre-swim values within about 1 h following the swimming period.

Adrenaline injection in group I fish produced marked elevation of both *P*<sub>DA</sub> and *P*<sub>VA</sub>. Cardiac output increased substantially as a result of an increase in *V*<sub>s</sub>, while *f*<sub>H</sub> remained largely unchanged. *R*<sub>gill</sub> decreased slightly, while changes in *R*<sub>sys</sub>, if any, were transient (see Fig. 4).

After treatment of the fish in group I with the  $\alpha$ -adrenoceptor antagonist prazosin (1 mg kg<sup>-1</sup>) there was a decrease in resting *P*<sub>DA</sub> due to a decrease in *R*<sub>sys</sub> (Table 1). Stroke volume increased by 73.3 %. Only seven fish were able to swim after the prazosin

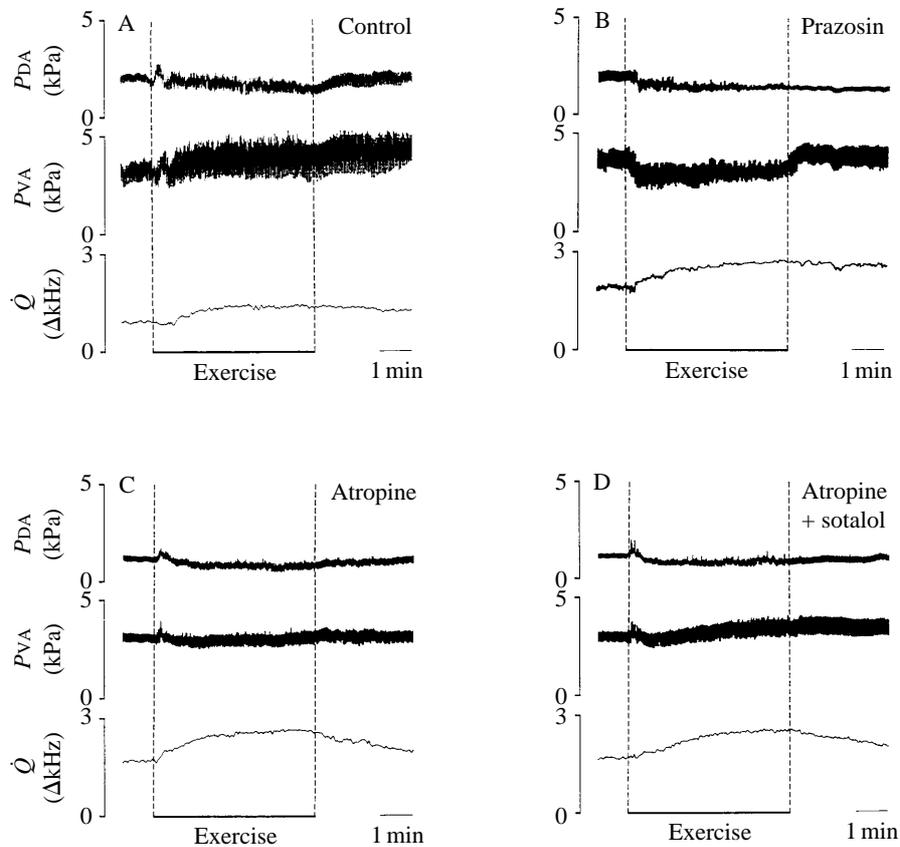


Fig. 1. Original tracings of  $P_{VA}$ ,  $P_{DA}$  and  $\dot{Q}$  from *Pagothenia borchgrevinki* during exercise in an untreated control fish (A) and fish treated with prazosin (B), atropine (C) and atropine+sotalol (D).

Table 1. Percentage changes in resting cardiovascular variables induced by treatment with the antagonists prazosin and enalapril (group I) or atropine and sotalol (group II)

|            | Group I           |                     | Group II          |                   |
|------------|-------------------|---------------------|-------------------|-------------------|
|            | Prazosin          | Prazosin +enalapril | Atropine          | Atropine +sotalol |
| $P_{DA}$   | $-26.3 \pm 4.0^*$ | $-30.1 \pm 12.9^*$  | $-13.4 \pm 7.7^*$ | $-10.4 \pm 5.2$   |
| $P_{VA}$   | $-10.5 \pm 6.2$   | $-15.6 \pm 8.0$     | $-6.9 \pm 9.8$    | $2.7 \pm 5.0$     |
| $\dot{Q}$  | $47.3 \pm 17.8$   | $21.0 \pm 5.3^*$    | $38.6 \pm 11.0^*$ | $1.4 \pm 10.4$    |
| $f_H$      | $-10.1 \pm 8.8$   | $9.3 \pm 8.9$       | $50.7 \pm 11.7^*$ | $-11.8 \pm 1.3^*$ |
| $R_{sys}$  | $-45.8 \pm 7.2^*$ | $-44.0 \pm 8.4^*$   | $-35.7 \pm 5.7^*$ | $-7.8 \pm 7.6$    |
| $R_{gill}$ | $25.7 \pm 26.0$   | $3.8 \pm 13.2$      | $23.1 \pm 35.6$   | $50.2 \pm 21.4$   |
| $V_s$      | $73.3 \pm 27.1^*$ | $15.1 \pm 9.6$      | $-3.8 \pm 9.8$    | $15.6 \pm 13.3$   |

Asterisks indicate statistically significant difference compared with control values ( $P < 0.05$ ). Values are mean  $\pm$  S.E.M. ( $N = 12$  in both groups).

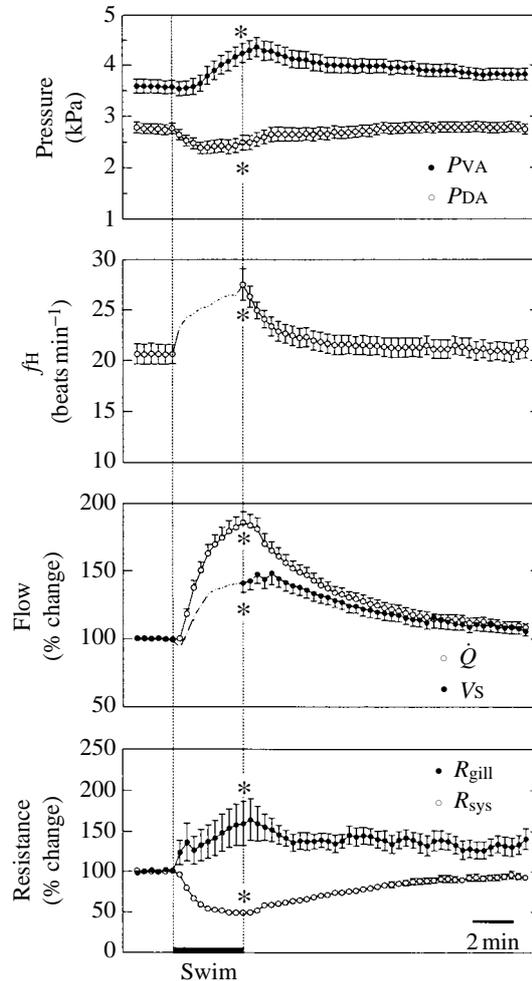


Fig. 2. Graphs showing the effects of 5 min of exercise (swimming at  $0.8\text{--}1$  body lengths  $\text{s}^{-1}$ ) on blood pressures ( $P_{VA}$  and  $P_{DA}$ ), heart rate ( $f_H$ ), cardiac output ( $\dot{Q}$ ), stroke volume ( $VS$ ) and vascular resistance of the gills ( $R_{gill}$ ) and systemic vasculature ( $R_{sys}$ ). Owing to the variability in the signal triggering the tachometer during the swimming exercise,  $f_H$  and  $VS$  are shown as dashed lines only during this period. Data are presented as mean  $\pm$  S.E.M. ( $N=24$ ). Asterisks show statistically significant changes between values at the end of the swim period compared with resting values ( $P<0.05$ ).

treatment. In these fish, there was a decrease in  $P_{DA}$  similar to that found in the control group. In contrast to the control fish, there was an initial drop in  $P_{VA}$ , which, however, returned towards pre-swim values before the swim had ended. Cardiac output increased, entirely because of an increase in  $f_H$ , since  $VS$  did not change from its new, high level. Recovery of  $R_{sys}$  and  $P_{DA}$  following exercise was somewhat slower than in the drug-free fish (Fig. 3).

Following prazosin treatment, a second adrenaline injection still produced increases in

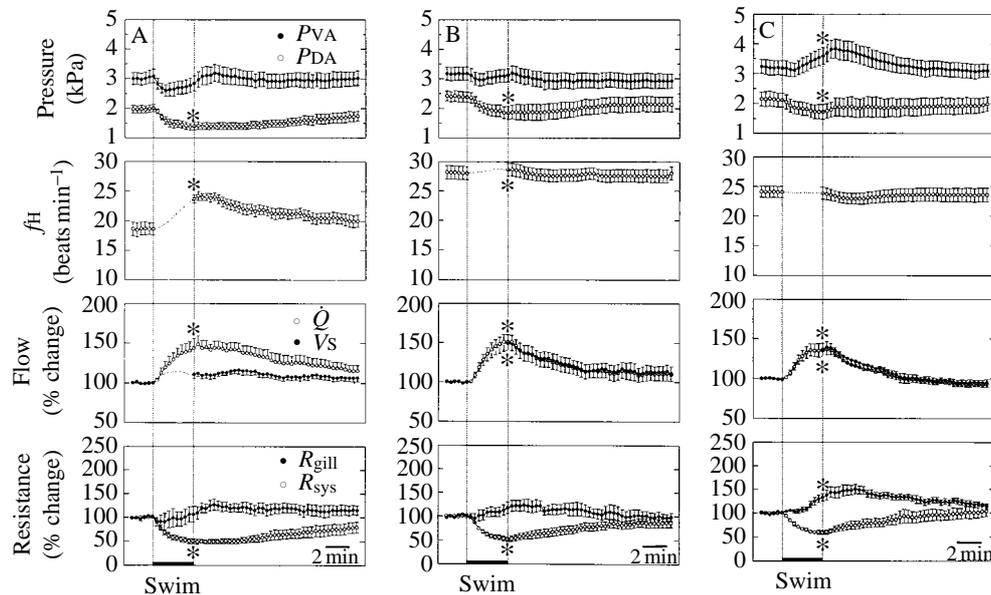


Fig. 3. Graphs showing the effects of 5 min of exercise (swimming at  $0.8\text{--}1$  body lengths  $\text{s}^{-1}$ ) on blood pressures ( $P_{VA}$  and  $P_{DA}$ ), heart rate ( $f_H$ ), cardiac output ( $\dot{Q}$ ), stroke volume ( $V_s$ ) and vascular resistance of the gills ( $R_{gill}$ ) and systemic vasculature ( $R_{sys}$ ). Owing to the variability in the signal triggering the tachometer during the swimming exercise,  $f_H$  and  $V_s$  are shown as dashed lines only during this period. Data are presented as mean  $\pm$  S.E.M. for  $N=7$  (prazosin-treated, A),  $N=10$  (atropine-treated, B) and  $N=8$  (atropine+sotalol-treated, C). Asterisks show statistically significant changes between values at the end of the swim period compared with resting values ( $P<0.05$ ). Note that flows and vascular resistances are expressed as percentage changes from pre-exercise values. Changes in resting and exercise values induced by the antagonist treatments are summarized in Table 1.

$P_{DA}$  and  $P_{VA}$ , although they were less pronounced and they were associated with smaller increases in  $V_s$  and  $\dot{Q}$ . However,  $R_{sys}$  and  $R_{gill}$  did not change (Fig. 4).

Ang II produced consistent increases in  $R_{sys}$ , while  $R_{gill}$  decreased slightly. In fish that had not had any previous treatment with drugs (group II),  $P_{DA}$  and  $P_{VA}$  increased after Ang II injection ( $P_{DA}$  from  $2.79\pm 0.11$  to  $3.98\pm 0.14$  kPa;  $P_{VA}$  from  $3.72\pm 0.14$  to  $4.81\pm 0.16$  kPa) with peak pressure at 2–3 min (Fig. 5). This initial rise in  $P_{DA}$  and  $P_{VA}$  occurred without a change in  $\dot{Q}$  as bradycardia compensated for a rise in  $V_s$ . In the group I fish, the rise in pressure in response to Ang II was slowed somewhat (peak after more than 5 min), but here Ang II was given after pretreatment with a number of other drugs, including prazosin (Fig. 6).

The hypertensive effect of Ang I was abolished by pretreatment with the angiotensin converting enzyme (ACE) inhibitor enalapril. Ang I had a similar hypertensive effect to Ang II, but without the marked bradycardia associated with Ang II injection (Fig. 6). The pressor response was slower to develop. Both Ang I and Ang II injections caused an immediate increase in  $V_s$ .

A striking effect in the group II fish was the bradycardia induced by Ang II injection

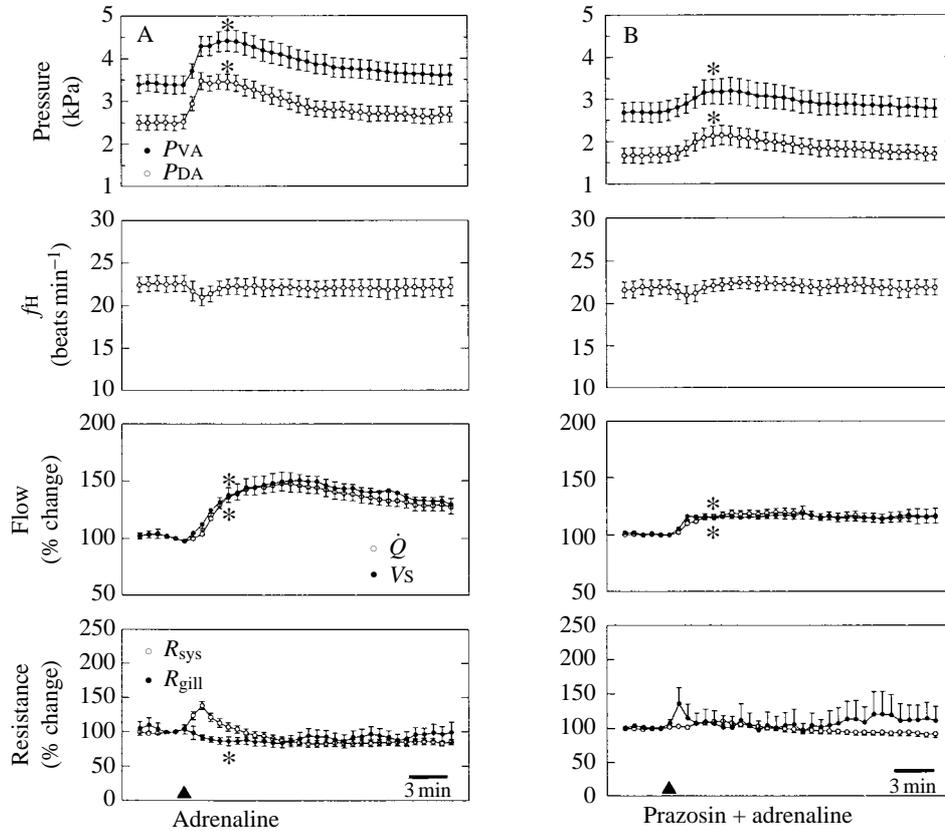


Fig. 4. Graphs showing the effects of adrenaline injection ( $5 \text{ nmol g}^{-1}$ ) on blood pressures ( $P_{VA}$  and  $P_{DA}$ ), heart rate ( $f_H$ ), cardiac output ( $\dot{Q}$ ), stroke volume ( $V_s$ ) and vascular resistance of the gills ( $R_{gill}$ ) and systemic vasculature ( $R_{sys}$ ). Data are presented as mean  $\pm$  S.E.M. for  $N=13$  (adrenaline control) and  $N=12$  (adrenaline after prazosin treatment). Asterisks show statistically significant changes between values at peak blood pressure response compared with pre-injection values ( $P<0.05$ ). Note that flows and vascular resistances are expressed as percentage changes from pre-exercise values. Changes in resting and exercise values induced by the prazosin treatment are summarized in Table 1.

(Fig. 5). Atropine injection changed this to a tachycardia,  $f_H$  being 35 % greater than the pre-Ang II injection values. Following atropine injection,  $f_H$  remained high and regular (Figs 3, 5). Atropine injection produced a substantial decrease in resting  $R_{sys}$  and, despite an increase in  $\dot{Q}$  (due to elevated  $f_H$ ),  $P_{DA}$  decreased (Table 1).

The increase in  $P_{VA}$  and  $R_{gill}$  observed during swimming in the control animals was absent in atropinized fish. The increase in  $\dot{Q}$  was due almost entirely to an increase in  $V_s$ , although a small increase in  $f_H$  did occur (Fig. 3).

When the atropinized fish were treated with sotalol, resting  $f_H$  fell (Table 1). Sotalol restored the exercise-induced increase in  $P_{VA}$ , in association with an increase in  $R_{gill}$  (Fig. 3).

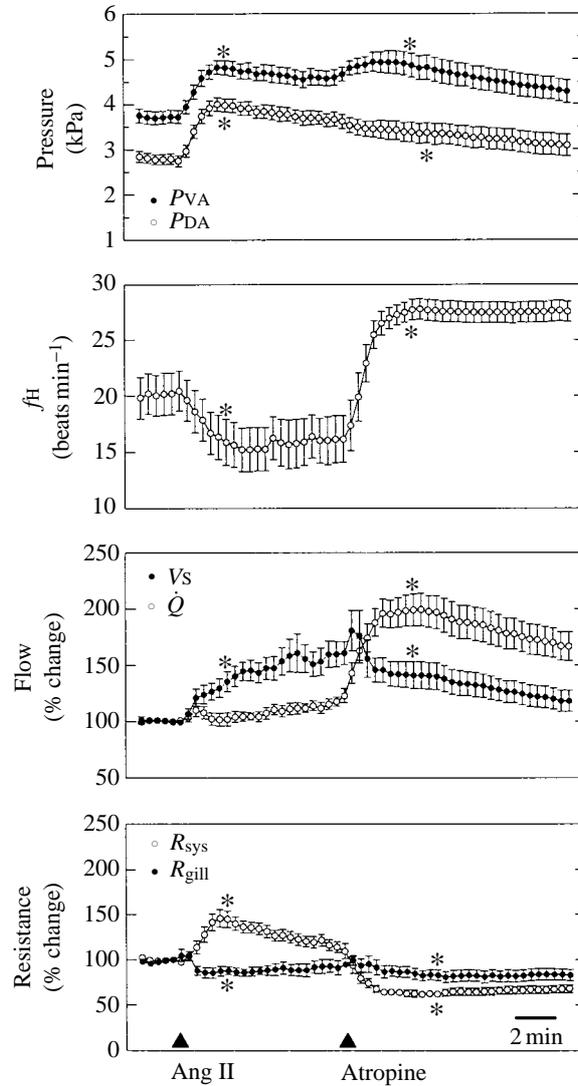


Fig. 5. Graphs showing the effects of angiotensin II (Ang II) injection ( $1 \text{ nmol g}^{-1}$ ), followed by atropine ( $1 \text{ mg kg}^{-1}$ ), on blood pressures ( $P_{VA}$  and  $P_{DA}$ ), heart rate ( $f_H$ ), cardiac output ( $\dot{Q}$ ), stroke volume ( $V_s$ ) and vascular resistance of the gills ( $R_{\text{gill}}$ ) and systemic vasculature ( $R_{\text{sys}}$ ). Data are presented as mean  $\pm$  S.E.M. ( $N=10$ ). Asterisks show statistically significant changes between values at peak blood pressure response (Ang II effect) or peak heart rate response (atropine effect) compared with pre-injection values ( $P<0.05$ ).

An incidental observation of spleen status was made during the later part of this study in the freshly killed atropinized and atropine/sotalol-treated fish. These fish showed dark blood-engorged spleens weighing  $0.68 \pm 0.17\%$  of body mass ( $N=4$ ).

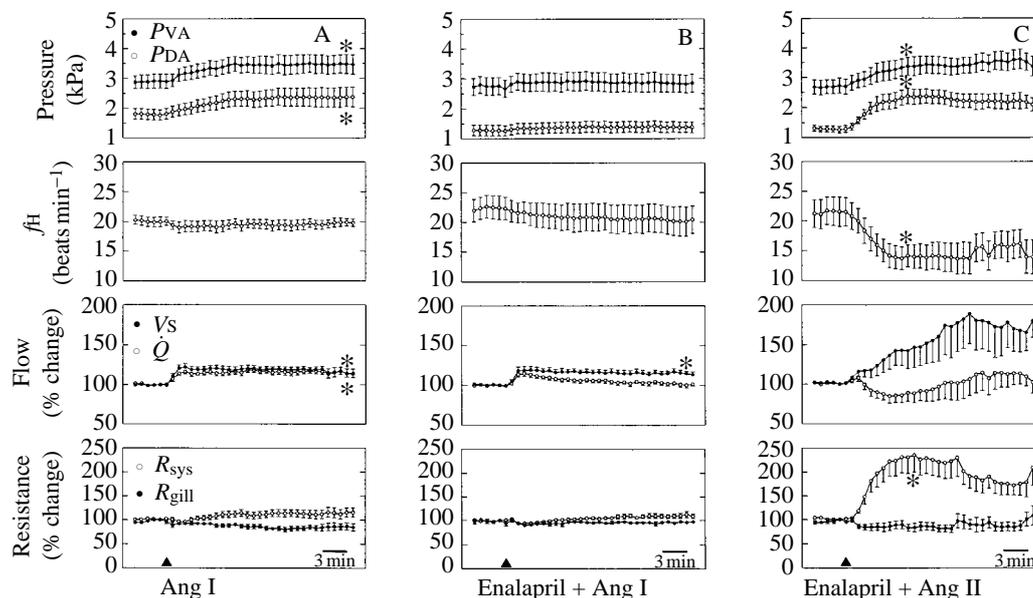


Fig. 6. Graphs showing the effects of injection of angiotensin I (Ang I;  $1 \text{ nmol g}^{-1}$ ; A), angiotensin I after enalapril treatment (Ang I;  $1 \text{ nmol g}^{-1}$ ; B), angiotensin II after enalapril treatment (Ang II;  $1 \text{ nmol g}^{-1}$ ; C) on blood pressures ( $P_{VA}$  and  $P_{DA}$ ), heart rate ( $f_H$ ), cardiac output ( $\dot{Q}$ ), stroke volume ( $V_s$ ) and vascular resistance of the gills ( $R_{gill}$ ) and systemic vasculature ( $R_{sys}$ ). Data are presented as mean  $\pm$  S.E.M. ( $N=7$ ). Asterisks show statistically significant changes between values at peak blood pressure response (Ang I effect) or peak heart rate response (atropine effect) compared with pre-injection values ( $P < 0.05$ ). Note that flows and vascular resistances are expressed as percentage changes from pre-exercise values. Changes in resting and exercise values induced by the enalapril treatment are summarized in Table 1.

## Discussion

The surgical procedure used in the present study involves occlusion of one gill arch to allow access to both afferent and efferent branchial arteries for blood pressure recording. The procedure is not vastly different from that used in a previous study of the same species under otherwise nearly identical conditions, yet the heart rate appears to be substantially higher in the present work (mean  $f_H$  was  $20.6 \text{ beats min}^{-1}$  compared with  $11.3 \text{ beats min}^{-1}$ ; Axelsson *et al.* 1992). One difference between the two studies, apart from the slight difference in cannulation procedure, was the use of MS222 (tricaine) (Axelsson *et al.* 1992) or benzocaine (present work) anaesthesia. In a parallel study of the benthic nototheniid *Trematomus (Pagothenia) bernacchi*, a similarly high  $f_H$  was observed, compared with the values obtained by Axelsson *et al.* (1992). Switching to MS222 anaesthesia had no effect on the high heart rate in that species. The other difference between the two studies was that the fish in the present study were held in the aquarium system in Christchurch for a considerably shorter period (less than 3 weeks in this study compared with more than 6 weeks in the previous). The range of heart rates

observed in the present study was large, indicating that, in *P. borchgrevinki*, small differences in experimental protocol may cause variations in the cholinergic tonus regulating  $f_H$  (Axelsson *et al.* 1992). Any of these three factors (surgical procedure, anaesthesia and holding time), alone or in combination, may be responsible for the discrepancy in the resting  $f_H$  but, until further analysis can be made, we cannot offer a final explanation to the observed difference.

Swimming produced an increase in  $\dot{Q}$  resulting from both tachycardia and increased  $V_s$ . Despite this, swimming fish showed a clear and remarkably consistent decrease in  $P_{DA}$  resulting from a marked drop in  $R_{sys}$ . All swimming episodes produced a drop in  $R_{sys}$ . The  $\alpha$ -adrenoceptor antagonist prazosin lowered resting  $P_{DA}$ , demonstrating the existence of an  $\alpha$ -adrenergic tonus affecting resting systemic blood pressure in borchs. Atropine also reduced  $R_{sys}$ , indicating a cholinergic vasomotor tonus, the function of which is clearly in need of further investigation. With more than one possible controlling system affecting systemic resistance, it is perhaps not surprising that  $R_{sys}$  fell even further after exercise in both the prazosin- and atropine-treated fish.

Prazosin reduced the increases in  $R_{gill}$  and  $V_s$  seen during exercise. In addition to the well-known  $\beta$ -adrenoceptor-mediated vasodilation of the arterio-arterial pathway in teleosts, adrenergic nerve stimulation and exogenous catecholamines produce an  $\alpha$ -adrenoceptor-mediated vasoconstriction of the arterio-venous pathway and efferent lamellar arterioles (Nilsson and Pettersson, 1981; Pettersson, 1983; Laurent, 1984; Nilsson, 1984). This vasoconstrictor mechanism appears to be present in borchs. The significance of this  $\alpha$ -adrenoceptor-mediated vasoconstriction is not fully understood, but an increased lamellar recruitment has been suggested which, in turn, may facilitate oxygen uptake (Pettersson and Johansen, 1982; Pettersson, 1983).

In addition to the adrenoceptor-mediated vasomotor control of the branchial vasculature, there is good histochemical evidence from several teleost species for cholinergic vasoconstrictor innervation of the sphincter at the base of the efferent filamental artery (Bailly and Dunel-Erb, 1986; Dunel-Erb *et al.* 1989). Physiological studies corroborate the structural findings, showing a functional cholinergic vasoconstrictor control of the filamental sphincters (Smith, 1977, 1978; Pettersson and Nilsson, 1979; Nilsson and Pettersson, 1981; Nilsson, 1984). In the present study, atropine reduced or abolished the branchial vasoconstriction seen during exercise, suggesting the presence of a similar cholinergic vasoconstrictor control in borchs. A  $\beta$ -adrenoceptor-mediated vasodilator mechanism may also be present, since the branchial vasoconstrictor response to exercise, which was abolished in atropinised fish, returned when they were treated with the  $\beta$ -adrenoceptor antagonist sotalol. However, treatment with prazosin and adrenaline did not reduce  $R_{gill}$ , which may indicate that there are few  $\beta$ -adrenoceptors in the gill. It would appear that the branchial vasculature of the borchs is mainly influenced by cholinergic and  $\alpha$ -adrenergic vasoconstrictor mechanisms. In the present study, we did not test the effects of adrenaline after  $\beta$ -adrenoceptor blockade, but the apparent dominance of  $\alpha$ -adrenoceptor mechanisms over  $\beta$ -adrenoceptor effects on the cardiovascular system of these cold-adapted fish is interesting. In teleost fish from temperate latitudes,  $\beta$ -adrenoceptor-mediated responses are dominant in summer, while  $\alpha$ -adrenoceptor-mediated responses are relatively more important in winter (Randall and Perry, 1992).

Adrenaline injection produced marked increases in  $P_{VA}$ ,  $P_{DA}$  and  $\dot{Q}$ .  $f_H$  remained largely unaffected, indicating that the change in  $\dot{Q}$  was due entirely to an increase in  $V_S$ . In contrast to most other teleosts and, indeed, to most vertebrates, the hypertension produced by adrenaline was caused almost entirely by an elevated  $\dot{Q}$ , rather than by increased vascular resistance. After prazosin treatment, part of the blood pressure response to adrenaline persisted, and the main action of prazosin appeared to be to weaken the effect of adrenaline on  $V_S$ . It is not clear whether the action of prazosin on the heart is due to nonspecific effects or whether the adrenergic control of the borch heart involves  $\alpha$ -adrenoceptors.

The renin-angiotensin system has been shown to possess strong effects on blood pressure in teleost fish and may be a major 'anti-drop' factor in teleost blood pressure control (Taylor, 1977; Bailey and Randall, 1980; Nishimura and Bailey, 1982; Gray and Brown, 1985; Olson, 1992; Platzack *et al.* 1993). As in other vertebrates, Ang II acts directly on the teleost vasculature and a substantial part of its vasomotor effect may be caused by secondary release of catecholamines (Nishimura *et al.* 1978; Carroll, 1981; Carroll and Opdyke, 1982; Olson, 1992). Ang II produced a consistent increase in both  $P_{VA}$  and  $P_{DA}$  as a result of a dramatic elevation in  $R_{sys}$ . In the experiments in group I, only the direct effects of angiotensin were recorded, since the  $\alpha$ -adrenoceptors had been blocked by prazosin.

The blood pressure rise after Ang I injection suggests the presence of endogenous angiotensin converting enzyme (ACE), which is necessary for the conversion of Ang I to the vasoactive Ang II. Treatment with the ACE inhibitor enalapril impaired or abolished the effects of Ang I, while Ang II was still active. Treatment with Ang I did not produce the large increase in  $R_{sys}$  and the bradycardia seen in response to Ang II. The slow development of pressure following Ang I injection indicates that Ang II was being produced fairly slowly, allowing other mechanisms to keep  $R_{sys}$  and  $f_H$  constant. The 10–20% increase in  $V_S$  following injection of a number of drugs, including Ang I and enalapril, may well be an artefact of volume loading (Figs 4 and 6). It can, therefore, be concluded that *P. borchgrevinki* possess vital elements of a renin-angiotensin system and that these mechanisms are part of the system controlling blood pressure in the borchs.

In some of the fish in group II, the bradycardia observed after Ang II injection was spectacular, with  $f_H$  reaching levels as low as 3–4 beats  $\text{min}^{-1}$ . The bradycardia was due to a cholinergic vagal barostatic reflex, since  $f_H$  increased dramatically after atropine treatment (Fig. 3). The marked barostatic reflex was not observed when adrenaline was used to increase blood pressure, where it is possible that the inhibitory effect of a vagal reflex was offset by a direct adrenergic stimulation of the heart. Certainly, sotalol treatment reduced heart rate in atropinised fish.

Incidental observation of spleen status in four freshly killed atropine- and atropine/sotalol-treated fish, showed a mean spleno-somatic index of  $0.68 \pm 0.17\%$ . This is somewhat higher than that observed in a previous systematic study ( $0.50 \pm 0.21\%$  in resting controls; Franklin *et al.* 1993). In contrast to that of other vertebrates, the teleost spleen is controlled in part by cholinergic excitatory nerve fibres (cf. Nilsson and Grove, 1974). In view of this, a swelling of the spleen due to sequestering of erythrocytes could be expected to occur after atropine treatment. However, no systematic evaluation of

atropine effects on the borch spleen was made in the present study, and the observed increase needs further clarification.

We conclude that *P. borchgrevinki* regulates its blood pressure more through modulation of cardiac performance (cardiac output) than by changing vascular resistance. This may be a consequence of total vascular resistance being unusually low in borchs compared with most teleost species (Axelsson *et al.* 1992). The scope of heart rate control is large: from as low as 3–4 beats min<sup>-1</sup> in individual fish during hypertensive bradycardia to about 28 beats min<sup>-1</sup> after atropine treatment. During exercise, a consistent decrease in  $R_{\text{sys}}$  occurred, possibly because of a reduction in a cholinergic as well as an  $\alpha$ -adrenergic tonus or because of an exercise-related ‘active hyperaemia’ (Randall and Daxboeck, 1982). The prerequisites for angiotensin-related vasomotor control mechanisms appear to be present.

This work was supported by grants from the New Zealand Antarctic Programme (NZAP), the Swedish Natural Science Research Council (NFR) and the Swedish Forestry and Agriculture Research Council (SJFR). We want to express our most cordial thanks to the staff of Scott Base, Antarctica, for their friendship and support.

### References

- AXELSSON, M., DAVISON, W., FORSTER, M. E. AND FARRELL, A. P. (1992). Cardiovascular responses of the red-blooded Antarctic fishes, *Pagothenia bernacchi* and *Pagothenia borchgrevinki*. *J. exp. Biol.* **167**, 179–201.
- AXELSSON, M., FARRELL, A. P. AND NILSSON, S. (1990). Effects of hypoxia and drugs on the cardiovascular dynamics of the Atlantic hagfish, *Myxine glutinosa*. *J. exp. Biol.* **151**, 297–316.
- AXELSSON, M. AND FRITSCHÉ, R. (1991). Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod, *Gadus morhua*. *J. exp. Biol.* **158**, 181–198.
- AXELSSON, M. AND NILSSON, S. (1986). Blood pressure control during exercise in the Atlantic cod *Gadus morhua*. *J. exp. Biol.* **126**, 225–236.
- BAILEY, J. R. AND RANDALL, D. J. (1980). Renal perfusion pressure and renin secretion in the rainbow trout, *Salmo gairdneri*. *Can. J. Zool.* **59**, 1220–1226.
- BAILLY, Y. AND DUNEL-ERB, S. (1986). The sphincter of the efferent filament artery in teleost gills. I. Structure and parasympathetic innervation. *J. Morph.* **187**, 219–237.
- CAMERON, J. S. (1979). Autonomic nervous tone and regulation of heart rate in the gold fish, *Carassius auratus*. *Comp. Biochem. Physiol.* **63C**, 341–349.
- CARROLL, R. G. (1981). Vascular response of the dogfish and sculpin to angiotensin II. *Am. J. Physiol.* **240**, R139–R143.
- CARROLL, R. G. AND OPDYKE, D. F. (1982). Evolution of angiotensin II-induced catecholamine release. *Am. J. Physiol.* **243**, R65–R69.
- DAVISON, W., FORSTER, M. E., FRANKLIN, C. E. AND TAYLOR, H. H. (1988). Recovery from exhausting exercise in an Antarctic fish, *Pagothenia borchgrevinki*. *Polar Biol.* **8**, 167–171.
- DUNEL-ERB, S., BAILLY, Y. AND LAURENT, P. (1989). Neurons controlling the gill vasculature in five species of teleosts. *Cell Tissue Res.* **255**, 567–573.
- EGGINTON, S., TAYLOR, E. W., WILSON, R. W., JOHNSTON, I. A. AND MOON, T. W. (1991). Stress response in the Antarctic teleosts (*Notothenia neglecta* Nybelin and *N. rossii* Richardson). *J. Fish Biol.* **38**, 225–236.
- FARRELL, A. P. (1984). A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. *Can. J. Zool.* **62**, 523–536.
- FORSTER, M. E., FRANKLIN, C. E., TAYLOR, H. H. AND DAVISON, W. (1987). The aerobic scope of an Antarctic fish, *Pagothenia borchgrevinki* and its significance for metabolic cold adaptation. *Polar Biol.* **8**, 155–159.

- FRANKLIN, C. E., DAVISON, W. AND MCKENZIE, J. C. (1993). The role of the spleen during exercise in the Antarctic teleost, *Pagothenia borchgrevinki*. *J. exp. Biol.* **174**, 381–386.
- GRAY, C. J. AND BROWN, J. A. (1985). Renal and cardiovascular effects of angiotensin II in the rainbow trout, *Salmo gairdneri*. *Gen. comp. Endocr.* **59**, 375–381.
- HOLM, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70.
- LAURENT, P. (1984). Gill internal morphology. In *Fish physiology*, vol. X (ed. W. S. Hoar and D. J. Randall), pp. 73–183. Orlando: Academic Press.
- MACDONALD, J. A., MONTGOMERY, J. C. AND WELLS, R. M. G. (1987). Comparative physiology of Antarctic fishes. In *Advances in Marine Biology*, vol. 24 (ed. J. H. S. Blaxter and A. J. Southward), pp. 321–388. London: Academic Press.
- MACDONALD, J. A. AND WELLS, R. M. G. (1991). Viscosity of body fluids from Antarctic notothenioid fish. In *Biology of Antarctic Fish* (ed. G. di Prisco, B. Maresca and B. Tota), pp. 163–178. Berlin, Heidelberg, New York: Springer-Verlag.
- MONTGOMERY, J. C. AND WELLS, R. M. G. (1993). Recent advances in the ecophysiology of Antarctic notothenioid fishes: metabolic capacity and sensory performance. In *Fish Ecophysiology* (ed. J. C. Rankin and F. B. Jensen) pp. 341–374. London: Chapman and Hall.
- NILSSON, S. (1984). Innervation and pharmacology of the gills. In *Fish Physiology*, vol. XA (ed. W. S. Hoar and D. J. Randall), pp. 185–227. Orlando: Academic Press.
- NILSSON, S. AND GROVE, D. J. (1974). Adrenergic and cholinergic innervation of the spleen of the cod, *Gadus morhua*. *Eur. J. Pharmac.* **28**, 135–143.
- NILSSON, S. AND HOLMGREN, S. (1992). Cardiovascular control by purines, 5-hydroxytryptamine and neuropeptides. In *Fish Physiology*, vol. XIIB (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 301–341. San Diego: Academic Press.
- NILSSON, S. AND PETTERSSON, K. (1981). Sympathetic nervous control of blood flow in the gills of the Atlantic cod, *Gadus morhua*. *J. comp. Physiol.* **144**, 157–163.
- NISHIMURA, H. AND BAILEY, J. R. (1982). Intrarenal renin–angiotensin system in primitive vertebrates. *Kidney int.* **22**, S185–S192.
- NISHIMURA, H., NORTON, V. M. AND BUMPUS, F. M. (1978). Lack of specific inhibition of angiotensin II in eels by angiotensin antagonists. *Am. J. Physiol.* **235**, H95–H103.
- OLSON, K. R. (1992). Blood and extracellular fluid volume regulation: Role of the renin–angiotensin, kallikrein–kinin systems and atrial natriuretic peptides. In *Fish Physiology*, vol. XIIB (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 135–254. San Diego: Academic Press.
- PETTERSSON, K. (1983). Adrenergic control of oxygen transfer in perfused gills of the cod, *Gadus morhua*. *J. exp. Biol.* **102**, 327–335.
- PETTERSSON, K. AND JOHANSEN, K. (1982). Hypoxic vasoconstriction and the effects of adrenaline on gas exchange efficiency in fish gills. *J. exp. Biol.* **97**, 263–272.
- PETTERSSON, K. AND NILSSON, S. (1979). Nervous control of the branchial vascular resistance of the Atlantic cod, *Gadus morhua*. *J. comp. Physiol.* **129**, 179–183.
- PLATZACK, B., AXELSSON, M. AND NILSSON, S. (1993). The renin–angiotensin system in blood pressure control during exercise in the cod, *Gadus morhua*. *J. exp. Biol.* **180**, 253–262.
- RANDALL, D. J. AND DAXBOECK, C. (1982). Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Can. J. Zool.* **60**, 1135–1140.
- RANDALL, D. J. AND PERRY, S. F. (1992). Catecholamines. In *Fish Physiology*, vol. XIIB (ed. W. S. Hoar, D. J. Randall and A. P. Farrell) pp. 255–300. San Diego, Academic Press.
- RANDALL, D. J. AND STEVENS, E. D. (1967). The role of adrenergic receptors in cardiovascular changes associated with exercise in salmon. *Comp. Biochem. Physiol.* **21**, 415–424.
- SMITH, D. G. (1977). Sites of cholinergic vasoconstriction in trout gills. *Am. J. Physiol.* **233**, R222–R229.
- SMITH, D. G. (1978). Neural regulation of blood pressure in rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* **56**, 1678–1683.
- SMITH, D. G., NILSSON, S., WAHLQVIST, I. AND ERIKSSON, B.-M. (1985). Nervous control of the blood pressure in the Atlantic cod, *Gadus morhua*. *J. exp. Biol.* **117**, 335–347.
- SUNDIN, L. AND NILSSON, S. (1992). Arterio-venous branchial blood flow in the Atlantic cod *Gadus morhua*. *J. exp. Biol.* **165**, 73–84.
- TAYLOR, A. A. (1977). Comparative physiology of the renin–angiotensin system. *Fedn Proc. Fedn Am. Socs exp. Biol.* **36**, 1776–1780.