

THE RENIN-ANGIOTENSIN SYSTEM IN BLOOD PRESSURE CONTROL DURING EXERCISE IN THE COD *GADUS MORHUA*

BJÖRN PLATZACK, MICHAEL AXELSSON and STEFAN NILSSON*

*Department of Zoophysiology, University of Göteborg, Medicinaregatan 18,
S-413 90 Göteborg, Sweden*

Accepted 22 March 1993

Summary

The effects of angiotensin I, angiotensin II and exercise on ventral and dorsal aortic blood pressure and heart rate were investigated in the Atlantic cod, *Gadus morhua*. Both angiotensins produced a marked increase in blood pressure. After injection of the angiotensin-converting enzyme (ACE) inhibitor enalapril, both ventral and dorsal blood pressures decreased significantly and the effect of angiotensin I was abolished. This demonstrates that ACE activity is necessary for conversion of angiotensin I to angiotensin II in the cod, as in other teleosts investigated, and provides a tool for further study of angiotensin function.

During swimming exercise at 2/3 body lengths per second, ventral and dorsal aortic blood pressures increased, but this exercise hypertension was absent in fish pretreated with the α -adrenoceptor antagonist prazosin. Instead, an increase in both ventral and dorsal aortic blood pressures occurred immediately after the exercise period. This post-exercise hypertension could be abolished by injection of enalapril, suggesting that the angiotensin system is the responsible 'anti-drop' factor activated in the absence of a functional adrenergic vasomotor control.

We conclude that the angiotensin system provides a major contribution to the resting blood pressure regulation in the cod, and that this system can be activated to offset a decrease in arterial blood pressure.

Introduction

The adrenergic control of cardiovascular functions in teleost fish during exercise has received some attention. During exercise, there is an increase in cardiac output [both heart rate (f_H) and stroke volume], while the effects on ventral and dorsal aortic blood pressures (P_{VA} and P_{DA}) appear to vary between species. Thus, the increase in cardiac output is offset in the rainbow trout by a reduction in systemic vascular resistance (Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982), whereas in the cod, vascular resistance remains largely unchanged during exercise and both blood pressures increase (Axelsson and Nilsson, 1986; see also Jones and Randall, 1978; Butler, 1986). Studies of circulatory

*Author for correspondence.

events during exercise in the cod show that injections of the adrenergic neurone-blocking agent bretylium abolish the exercise-induced hypertension, and that the adrenergic innervation of the systemic vascular beds is of paramount importance in maintaining vascular resistance, and therefore blood pressure, during exercise (Axelsson and Nilsson, 1986).

In fish in which the adrenergic vasoconstrictor control by both nerves and a possible contribution by circulating catecholamines had been blocked by combined treatment with bretylium and the α -adrenoceptor antagonist phentolamine, there was a marked increase in blood pressure starting at the end of the exercise period (Axelsson and Nilsson, 1986). Similar observations were made in toads (*Bufo marinus*) subjected to a similar adrenergic blockade (Wahlqvist and Campbell, 1988), but no final explanation for the phenomenon has been offered.

Of the possible non-adrenergic, non-cholinergic control mechanisms that could be implicated in the observed post-exercise hypertension, the renin-angiotensin system is one of the best known. Several functions of this system have been demonstrated in teleost fish, and components of the system include the angiotensin-converting enzyme responsible for hydrolysis of angiotensin I (Ang I) to the vasoactive angiotensin II (Ang II) (Olson *et al.* 1986, 1987, 1989; Olson, 1992). In fish, a role of the renin-angiotensin system in blood pressure regulation has been postulated (Taylor, 1977; Bailey and Randall, 1980; Nishimura and Bailey, 1982; Gray and Brown, 1985; Olson, 1992).

The purpose of this work was to examine the hypothesis that the renin-angiotensin system is responsible for the post-exercise hypertension observed after blockade of the adrenergic vasomotor system in the cod.

Materials and methods

Atlantic cod, *Gadus morhua* L., of either sex, with a body mass of 500–800g and length of 37–42cm were used. The fish were captured off the west coast of Sweden and kept in well-aerated, recirculating seawater at 10–12°C for 2–10 days before surgery. The animals were not fed while in captivity. The study was performed from March to May.

Surgical procedures

Fish were anaesthetized using MS-222 (3-aminobenzoic ethylester methanesulphonate; SIGMA) in seawater (100mg l⁻¹) until breathing movement ceased. They were then transferred to an operating table where seawater containing 50mg l⁻¹ MS-222 was continuously passed over the gills during surgery.

In two groups of eight fish, a polyethylene cannula (PE 50) filled with heparinized (50–100 i.u.ml⁻¹) 0.9% NaCl was occlusively inserted into the afferent branchial artery in the third gill arch for measurement of ventral aortic blood pressure (*P*_VA) and heart rate (*f*_H). These groups were used for experiments to establish the selectivity of the antagonists/inhibitors used (see later).

In a third group of eight fish, a cannula was occlusively inserted into the afferent branchial artery in the third gill arch for measurement of *P*_VA and *f*_H, and for injections of drugs. A similar cannula was inserted into the efferent branchial artery in the same gill

arch for measurement of dorsal aortic blood pressure (P_{DA}). Both cannulae were secured with skin sutures.

During the experiments, the cannulae were attached to Statham P23 pressure transducers connected to a Grass polygraph recorder system, model 79C. Calibration of blood pressure was made against a static water column. The heart rate was derived from the pulsative P_{VA} signal using a Grass 7P44 tachograph unit.

Experimental protocol

After surgery, the fish were transferred to a water channel similar to the one described by Axelsson and Nilsson (1986) and allowed to recover for 24h before any experiments were conducted. Water velocity in the channel was monitored using an impeller situated in the centre of the water flow. The water in the channel was kept at 10–12°C and was continuously replaced (at about 4 l min⁻¹) from the departmental seawater system.

Control experiments

To assess the selectivity of the angiotensin-converting enzyme (ACE) inhibitor enalapril and the α -adrenoceptor antagonist prazosin, experiments were conducted in which the 'wrong' antagonists were used. Thus, both angiotensins (Ang I and II) were tested in fish treated with prazosin, and the effects of adrenaline and Ang II injections were evaluated before and after enalapril. Prazosin was chosen, instead of the much-utilized α -adrenoceptor antagonist phentolamine, in an attempt to prolong the time of sufficient adrenoceptor blockade while avoiding the direct side effects of phentolamine (see Smith *et al.* 1985).

Eight fish from the first group were used for the prazosin control experiments. The fish were injected with Ang I (0.1 nmol kg⁻¹) and the effects recorded. Prazosin (1 mg kg⁻¹) was then injected and, after the cardiovascular parameters had stabilized (45–90 min), Ang I was again injected (0.1 nmol kg⁻¹) and recordings of the effects made.

Similarly, eight fish were used for control of enalapril selectivity. Adrenaline (5 nmol kg⁻¹) was injected and changes in blood pressure and heart rate were recorded. After the cardiovascular parameters had reached stable levels, a dose of Ang II (0.1 nmol kg⁻¹) was injected and the effects again recorded. Enalapril (1 mg kg⁻¹) was injected and, after a recovery period of 30–60 min, during which the blood pressure and heart rate stabilized, the adrenaline and Ang II injections were repeated and the cardiovascular parameters were recorded.

Effects of exercise and angiotensin I

Exercise experiments were performed on fish with both ventral ($N=8$) and dorsal ($N=7$) blood pressure cannulae. Before the start of exercise, injections of angiotensin I (Ang I, 0.1 nmol kg⁻¹) were made into resting fish and the cardiovascular parameters recorded.

The exercise protocol used was similar to that described by Axelsson and Nilsson (1986), and each experimental run was started with a control recording of the resting values of P_{DA} , P_{VA} and f_H . The water flow was started and adjusted to a velocity corresponding to 2/3 body lengths per second. Water flow was stopped after 8 min and recordings of P_{DA} , P_{VA} and f_H were made for a post-exercise period of 8 min.

The fish were then injected with the α -adrenoreceptor blocking agent prazosin (1mgkg^{-1}) and allowed to recover until cardiovascular parameters stabilized (45–90min). A second exercise run was then performed, as described earlier.

The fish were finally treated with enalapril, 1mgkg^{-1} , and allowed to recover until the cardiovascular parameters reached steady levels (30–60min). Ang I was injected to confirm the effectiveness of the enalapril treatment and, after the cardiovascular parameters had recovered, a final exercise period was performed. Similarly, adrenaline (5nmolkg^{-1}) was injected at the end of the third exercise run to confirm the α -receptor blockade.

Drugs

The following drugs were used in the experiments: adrenaline (Sigma); [Asn¹, Val⁵, Asn⁹]-angiotensin I, salmon, (Peninsula Laboratories Inc.); angiotensin II, human (Sigma); enalapril (Renitec, MSD) and prazosin (Sigma).

Data acquisition and statistics

Cardiovascular parameters were continuously recorded using the Grass polygraph, as described above. In addition, data were fed into a PC computer running AD/DATA (P. Thorén, Department of Physiology, University of Göteborg) for later retrieval and calculations. Mean values were created at 0.5–1min intervals from continuous sampling at 6samplesmin^{-1} , and data presented in the graphs show mean values \pm S.E.M. for N animals.

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

Results

Drug experiments

Several experiments were performed to establish the degree of selectivity of the ACE-blocking capacity of enalapril and the α -adrenoreceptor blockade caused by prazosin. Comparison of the effects of Ang I and Ang II on P_{VA} before and after prazosin showed a reduction in the blood pressure response in prazosin-treated fish of about 50% for Ang I and 30% for Ang II (Fig. 1A,B).

Enalapril caused a decrease in pre-injection values of both P_{VA} and P_{DA} (Fig. 2B,C). In addition, enalapril injection produced a decrease in mean ventral aortic blood pressure in one of the two sets of experiments (Fig. 1C). There were no major differences in the blood pressure increase produced by Ang II or by adrenaline after enalapril treatment (Fig. 1C,D), although the pre-injection pressures were generally lower in the enalapril-treated groups.

Neither Ang I nor enalapril showed any effects on f_H (Fig. 2A). Injection of Ang I (0.1nmolkg^{-1}) produced a marked increase in both P_{VA} and P_{DA} , and this effect was completely abolished by pre-injection of enalapril (1mgkg^{-1}) (Fig. 2B,C).

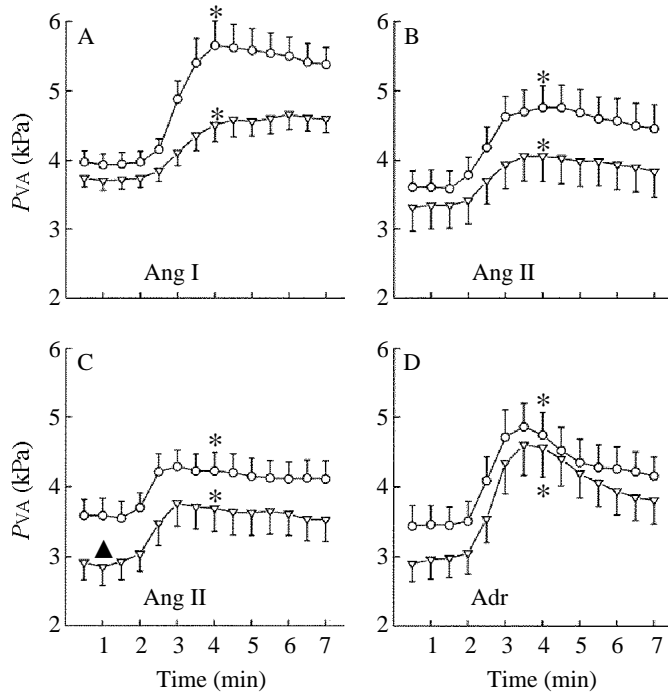


Fig. 1. Effects on ventral aortic blood pressure (P_{VA} ; $N=8$) of Ang I (A) and Ang II (B) injection (arrows), before (open circles) and after (open triangles) prazosin blockade, and Ang II (C) and adrenaline (Adr) (D) injection (arrows), before (open circles) and after (open triangles) enalapril blockade. Asterisks indicate significant differences compared with control values ($P<0.05$), the filled triangle indicates a significant difference in control values between different antagonist treatments ($P<0.05$). Vertical bars show S.E.M.

Exercise experiments

Heart rate (f_H) in the untreated animals increased rapidly at the start of the exercise period and fell quickly at the end of the exercise (Figs 3A and 4A). In animals treated with prazosin or prazosin and enalapril, f_H increased during exercise as in control fish, but return to the pre-exercise level was significantly slower than in the control fish ($P<0.05$). There were no significant differences in pre-exercise f_H between treatments (Fig. 4A).

The patterns of the blood pressure recordings in both P_{DA} and P_{VA} were similar. In the control group, exercise caused increased blood pressure, which returned towards pre-exercise values during the post-exercise period (Figs 3A and 4B,C).

In the prazosin-treated fish, both blood pressures decreased at the beginning of the exercise and remained below the pre-exercise value throughout the exercise period. At the end of the exercise, both pressures increased to values that were significantly ($P<0.05$) higher than the corresponding pre-exercise value (Figs 3B and 4B,C).

After the combined prazosin and enalapril treatment, pre-exercise mean blood pressures were significantly lower than in the control fish ($P<0.05$), and P_{DA} and P_{VA} decreased during exercise and did not recover during the post-exercise period (Figs 3C and 4B,C).

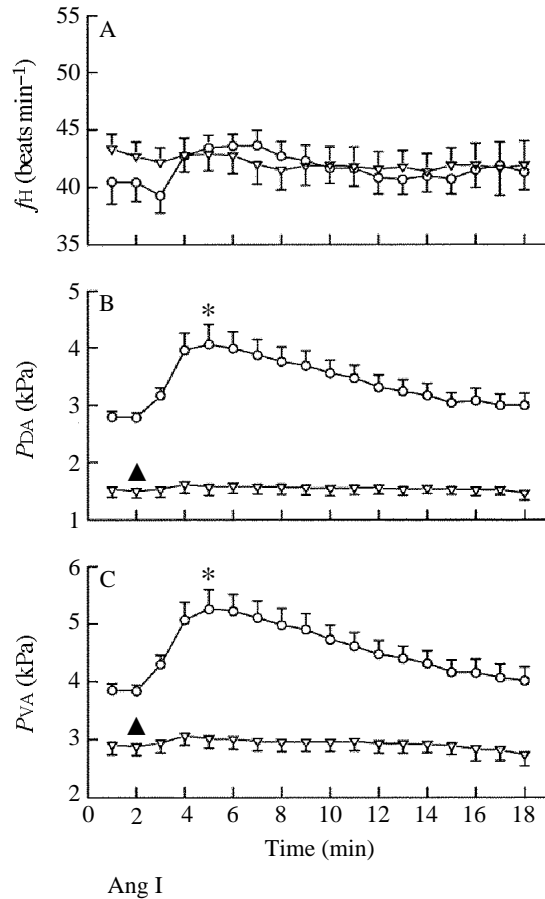


Fig. 2. Effects of Ang I on (A) heart rate (f_H ; $N=8$), (B) dorsal aortic blood pressure (P_{DA} ; $N=7$) and (C) ventral aortic blood pressure (P_{VA} ; $N=8$), in control fish (open circles) and after enalapril blockade (open triangles). Asterisks indicate significant differences compared to control (pre-injection) values ($P<0.05$), and filled triangles indicate significant differences between control values for untreated and enalapril-treated animals ($P<0.05$). Vertical bars show S.E.M.

Discussion

The cardiovascular parameters during both rest and exercise are comparable to those recorded in previous studies on *Gadus morhua* (for example, see Smith *et al.* 1985; Axelsson and Nilsson, 1986; Fritsche and Nilsson, 1990). The small reduction of P_{DA} seen after α -adrenoceptor blockade in other studies (for example, see Smith *et al.* 1985; Axelsson and Nilsson, 1986) was not evident in the present experiments, and the most striking effect on blood pressure was caused by enalapril inhibition of the angiotensin-converting enzyme. Post-exercise f_H remained high longer after treatment with prazosin than in control animals. This may reflect the lack of a major vagal drive to the heart in the absence of baroreceptor stimulation when the P_{DA} remained low during exercise.

Control experiments were performed to examine the selectivity of prazosin and

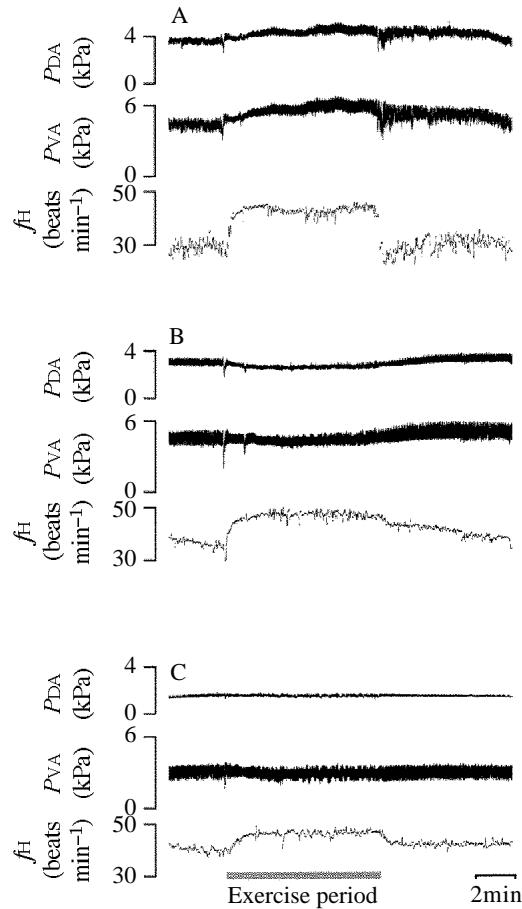


Fig. 3. Original tracings of dorsal aortic blood pressure (P_{DA}), ventral aortic blood pressure (P_{VA}) and heart rate (f_H) in cod during exercise (horizontal bar). (A) Control exercise period. (B) Fish treated with prazosin (1mgkg^{-1} bodymass). (C) Fish additionally treated with enalapril (1mgkg^{-1} bodymass). Note the hypertension, particularly the rise in P_{VA} , that starts at the end of the exercise period in B.

enalapril. From the experiments with the 'wrong' antagonists it is apparent that prazosin does, to a certain degree, impair the effect of the angiotensins. A reduction of the vasomotor effect of angiotensin after α -adrenoceptor blockade or reserpine treatment has also been observed in other teleosts, and probably reflects an ability of angiotensin to exert part of its vasomotor effect indirectly, by releasing catecholamines from adrenergic nerves (Nishimura *et al.* 1978; Carroll, 1981; Carroll and Opdyke, 1982; Olson, 1992).

The angiotensin-converting enzyme inhibitor enalapril had little or no effect on the responses to either Ang II or adrenaline. This means that the complete abolition of the effect of Ang I may be ascribed to a specific effect of enalapril on the conversion of Ang I to Ang II, in agreement with previous findings in fish (Nishimura *et al.* 1978; Nishimura and Bailey, 1982; Lipke and Olson, 1988; Olson *et al.* 1989; Olson, 1992). The marked

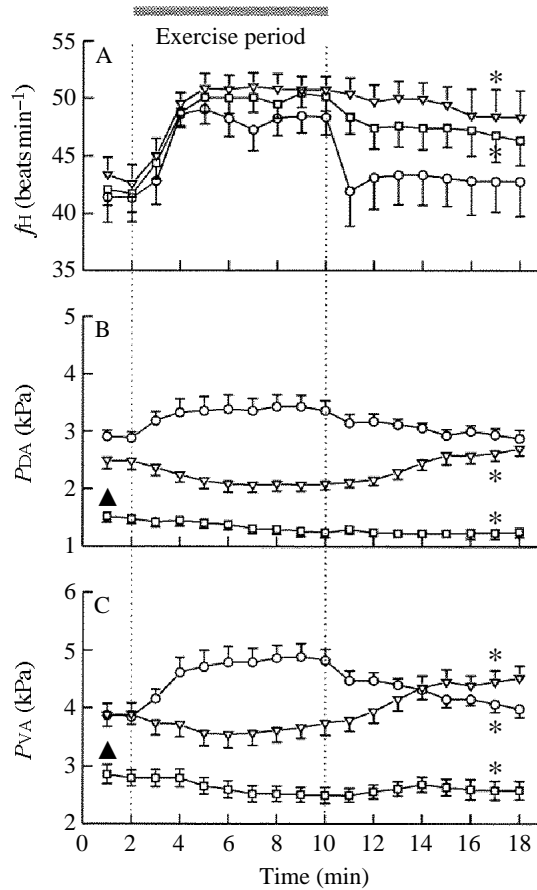


Fig. 4. Effects of exercise on (A) heart rate (f_H ; $N=8$), (B) dorsal aortic blood pressure (P_{DA} ; $N=7$) and (C) ventral aortic blood pressure (P_{VA} ; $N=8$) in control fish (open circles), after prazosin blockade (open triangles) and after combined prazosin and enalapril blockade (open squares). Asterisks indicate significant differences compared with control (pre-exercise) values ($P < 0.05$), filled triangles indicate significant differences between control values for untreated and combined prazosin+enalapril-treated animals ($P < 0.05$). Vertical bars show S.E.M.

blood pressure drop caused by enalapril alone suggests the existence of an angiotensin-tonus affecting arterial blood pressure, similar to the situation in rainbow trout (see, for example, Galardy *et al.* 1984; Lipke and Olson, 1990).

After prazosin treatment, both P_{VA} and P_{DA} decreased during exercise, similar to earlier observations using phentolamine as the α -adrenoceptor antagonist (Axelsson and Nilsson, 1986). This effect is probably due to the unmasking of an 'active hyperaemia' (Randall and Daxboeck, 1982), causing dilatation of the vasculature. The rise in blood pressure in prazosin-treated fish after the end of the exercise was recorded, but not commented upon, by Randall and Stevens (1967) using phenoxybenzamine as an α -adrenoceptor antagonist. The rise in blood pressure is also present in an amphibian, *Bufo marinus* (Wahlqvist and Campbell, 1988).

The present experiments, where the post-exercise hypertension is abolished by enalapril, are compatible with the view that the renin–angiotensin system is triggered by the exercise-induced fall in blood pressure and acts as ‘an anti-drop effector of blood pressure’ in fish (Olson, 1992).

It can thus be concluded that a renin–angiotensin system in cod works as a blood-pressure-regulating system during exercise. In the absence of an adrenergic vasomotor control, the cardiovascular control by angiotensins becomes clearly visible and produces the observed post-exercise hypertension.

We are greatly indebted to Dr Ken R. Olson for constructive criticism of the manuscript. The work was supported by grants from the Swedish Natural Science Research Council and the Forestry and Agriculture Research Council.

References

- 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.
- BUTLER, P. J. (1986). Exercise. In *Fish Physiology: Recent Advances* (ed. S. Nilsson and S. Holmgren), pp. 102–118. London, Canberra: Croom-Helm.
- 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.
- FRITSCH, R. AND NILSSON, S. (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Exp. Biol.* **48**, 153–160.
- FRITSCH, R. AND NILSSON, S. (1990). Autonomic nervous control of blood pressure and heart rate during hypoxia in the cod, *Gadus morhua*. *J. comp. Physiol.* **160B**, 287–292.
- GALARDY, R., PODHASKY, P. AND OLSON, K. R. (1984). Angiotensin-converting enzyme activity in tissues of the rainbow trout. *J. exp. Zool.* **230**, 155–158.
- GRAY, C. J. AND BROWN, J. A. (1985). Renal and cardiovascular effects of angiotensin II in the rainbow trout, *Salmo gairdneri*. *Gen. comp. Endocrinol.* **59**, 375–381.
- HOLM, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70.
- JONES, D. R. AND RANDALL, D. J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 425–501. New York: Academic Press.
- KICENIUK, J. W. AND JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.
- LIPKE, D. E. AND OLSON, K. R. (1988). Distribution of angiotensin-converting enzyme-like activity in vertebrate tissues. *Physiol. Zool.* **61**, 420–428.
- LIPKE, D. W. AND OLSON, K. R. (1990). Enzymes of the kallikrein–kinin system in rainbow trout. *Am. J. Physiol.* **258**, R501–R506.
- 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. 12B (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 135–254.
- OLSON, K. R., KULLMAN, D., NARKATES, A. J. AND OPARIL, S. (1986). Angiotensin extraction by trout tissues *in vivo* and metabolism by the perfused gill. *Am. J. Physiol.* **250**, R532–R538.
- OLSON, K. R., LIPKE, D. W., KULLMAN, D., EVAN, A. P. AND RYAN, J. W. (1989). Localization of angiotensin-converting enzyme in the trout gill. *J. exp. Zool.* **250**, 109–115.

- OLSON, K. R., LIPKE, D. W., MUNSHI, J. S. D., MOITRA, A., GHOSH, T. K., AHMAD, M., ROY, P. K., SINGH, O. N., NASAR, S. S. T., PANDEY, A., ODULEYE, S. O. AND KULLMAN, D. (1987). Angiotensin-converting enzyme in organs of air-breathing fish. *Gen. comp. Endocr.* **68**, 486–491.
- 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 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., NILSSON, S., WAHLQVIST, I. AND ERIKSSON, B-M. (1985). Nervous control of the blood pressure in the Atlantic cod, *Gadus morhua*. *J. exp. Zool.* **117**, 335–347.
- TAYLOR, A. A. (1977). Comparative physiology of the renin–angiotensin system. *Fedn Proc. Fedn Am. Socs exp. Biol.* **36**, 1776–1780.
- WAHLQVIST, I. AND CAMPBELL, G. (1988). Autonomic influences on heart rate and blood pressure in the toad, *Bufo marinus*, at rest and during exercise. *J. exp. Biol.* **134**, 377–396.