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CARDIOVASCULAR RESPONSES TO ADENOSINE IN THE ANTARCTIC FISH *PAGOTHENIA BORCHGREVINKI*

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Summary

We have investigated the effects of adenosine on the cardiovascular system of the Antarctic fish *Pagothenia borchgrevinki*. Continuous measurements of ventral and dorsal aortic blood pressures, heart rate (f_H) and ventral aortic blood flow (cardiac output, $\dot{Q}$) were made using standard cannulation techniques and a single-crystal Doppler flowmeter. On line measurements of arterial P_{O_2} were made using an oxygen electrode connected to an extracorporeal loop.

Adenosine (10 nmol kg^{-1}) and the specific A_1-receptor agonist N^6-cyclopentyladenosine (CPA) elicited biphasic changes in the branchial and systemic resistances. While there was an initial decrease in the branchial resistance followed by an increase, the opposite was true for the systemic response. The resistance changes were significantly attenuated by aminophylline (a P_1-receptor antagonist) and 8-cyclopentyltheophylline (CPT; an A_1-receptor antagonist). In addition, adenosine induced an aminophylline-sensitive decrease in the arterial P_{O_2}. The reduction was attenuated when pre-injection arterial P_{O_2} was low.

Adenosine and CPA also caused a marked reduction in f_H, with CPA being more potent. The bradycardia was blocked by aminophylline and CPT, demonstrating an involvement of A_1 receptors in this response.

Key words: teleost, *Pagothenia borchgrevinki*, purinergic, circulation, gill, heart, blood pressure, receptor.

Introduction

Our understanding of cardiovascular control mechanisms in teleost fish is over-dependent on studies of the rainbow trout *Oncorhynchus mykiss*. While based at Scott Base, Antarctica, we took the opportunity to investigate the effects of adenosine on the cardiovascular system of *Pagothenia borchgrevinki*. Antarctic notothenioids have the lowest vascular resistances recorded in teleosts, perhaps associated with the high viscosity of blood at sub-zero temperatures (Davison et al., 1997). Other 'unusual' features of their cardiovascular control include an increase in branchial vascular resistance in response to swimming (Axelsson et al., 1994), and a strong vasoconstrictor effect of serotonin associated with the presence of serotonin-containing cells within the gills (Sundin et al., 1998; Forster et al., 1998). Furthermore, the fish species around the Antarctic continent have been genetically isolated for 15–25 million years as a result of Antarctic convergence and bottom topography. This offers a unique opportunity to explore an evolutionarily distinct and cold-adapted cardiovascular control system.

Adenosine is formed following the breakdown of ATP, ADP and ultimately AMP in energy-deficient cells. Upon release, adenosine can act as a humoral agent and participate in cardiovascular regulation. Another source of adenosine is release during purinergic neurotransmission. The cardiovascular effects of adenosine are mediated by two P_1 receptors denoted A_1 and A_2. In mammals, A_2 receptors mediate vasodilatation, while stimulation of A_1 receptors induces a vasoconstriction (for reviews, see Olsson and Pearson, 1990; Shyrock and Belardinelli, 1997). The presence of P_1 receptors in the branchial circulation of teleosts has been proposed (Colin and Leray, 1979, 1981; Okafor and Oduleye, 1986), and in rainbow trout the excitatory P_1 receptor was recently identified as an A_1 receptor (Sundin and Nilsson, 1996). Excitatory A_1 and inhibitory A_2 receptors have also been identified in the ventral aorta of the dogfish *Squalus acanthias* (Evans, 1992). Since adenosine does not cause a branchial vasoconstriction in the hagfish *Myxine glutinosa* (Axelsson et al., 1990) and A_1 receptors have not yet been found in invertebrate nervous tissue (Siebenaller and Murray, 1986), it is possible that subclasses of A_1 and A_2 receptor first appeared in the elasmobranchs (Burnstock, 1996).

While there is evidence for co-release of ATP from cholinergic nerves in frogs (Silinsky and Redman, 1996), no evidence of purinergic neurotransmission has yet been found in fish. However, adenosine production has been documented

in rainbow trout (*Oncorhynchus mykiss*) gills (Leray et al., 1978) and hypoxic flounder (*Platichtys flesus*) hearts (Lennard and Huddart, 1989), which suggests a possible involvement of this nucleotide in cardiovascular control in fish. Although adenosine is likely to be a potent endogenous regulator of blood flow through fish gills during hypoxia, the physiological response of branchial vasoconstriction might impair gas exchange because Sundin and Nilsson (1996) found that adenosine reduced the functional respiratory surface area by having a constrictor effect on the distal vasculature in the gill filament, 'de-enlisting' lamellae. We have used pharmacological tools and measurements of key cardiovascular variables including ventral and dorsal aortic pressures, cardiac output, heart rate and arterial P_{O_2} to investigate changes in vascular resistance in *P. borchgrevinki* and their consequences for gas exchange.

Materials and methods

Animals

Borchs (*Pagothenia borchgrevinki* Boulenger) were caught on baited hooks through a hole cut in the sea ice of McMurdo Sound, Antarctica. They were transported back to Scott Base (New Zealand Antarctic Program), where they were held in plastic tanks at −1.3 °C. Some animals were flown to Christchurch, New Zealand, where a part of this study was undertaken. In Christchurch, the animals were held in plastic tanks in a closed-circuit aquarium system at 0 °C. Before use in experiments, they were allowed at least 2 days to recover from the effects of capture and at least 10 days to recover from transportation to Christchurch.

Surgery

Twenty-four fish with body masses ranging from 60 to 120 g were used in these studies. Animals were given 24 h to recover from surgery before measurements were started. The insertions of afferent and efferent branchial artery cannulae and the placement of a Doppler flow probe around the ventral aorta followed the procedures outlined by Axelsson et al. (1994), and the equipment used to record pressures and flows was also the same. A Minipuls 3 pump (Gilson, Villiers-le-Bel, France) pumped the blood through the extracorporeal loop at a rate of approximately 100 µl min^{-1}, with a total circulation time of approximately 2 min. The on-line electrode (type 16-73) and meter (type OM-4) for measurements of arterial oxygen partial pressure were manufactured by Microelectrodes Inc. (Londonderry, NH, USA). The electrode was submerged in the same experimental chamber as the fish, ensuring equal temperatures, and was zeroed using sodium sulphite solution in borate. Air-saturated sodium chloride solution (0.9 %) was used for calibration, and the electrode proved to be stable during the experimental period.

Experimental protocol

It was impossible using the current apparatus to measure both cardiovascular variables and oxygen partial pressures concomitantly. Initially, measurements of pressures and heart rate were made, and subsequently the cannulae were connected for continuous measurements of oxygen partial pressures. This allowed us to establish whether the ventral and dorsal aortic cannulae were patent and that the animals had recovered well from the surgery (animals with little or no beat-to-beat variations in heart rate were discarded because this is a sign of stress).

Pilot experiments using the agonist drugs were performed to determine a dose that gave clear and consistent submaximal effects. The doses of antagonists given were sufficient to block the effects of the agonists in rainbow trout (Sundin and Nilsson, 1996). The agonists were given as bolus injections into the dorsal aorta at 0.1 ml kg^{-1}, and the antagonists were given at 1 ml kg^{-1}. Control injections of 0.9 % NaCl were given to ensure that the vehicle and the volume of the injection did not produce significant cardiovascular responses. Drugs were injected *via* the efferent artery.

Series 1

This part of the study was carried out at Scott Base, Antarctica. At the start of each trial, resting values of cardiac output ($\dot{Q}$), ventral aortic blood pressure (P_{VA}), dorsal aortic blood pressure (P_{DA}), heart rate (f_H) and arterial P_{O_2} were recorded before injections of adenosine. To allow measurements of the effects on blood pressures, heart rate and arterial P_{O_2}, adenosine (10 nmol kg^{-1}) was injected twice before and twice after pre-treatment with aminophylline (a P_1 receptor antagonist). After subsequent treatment with aminophylline, the fish were left for at least 30 min to recover, or until the cardiovascular variables had stabilised, before the adenosine injections were repeated.

Series 2

This part of the study was carried out in Christchurch, New Zealand. In this group, a specific agonist and antagonist for the adenosine A_1 receptor were used to determine whether this receptor induced the cardiovascular responses produced by adenosine in series 1. This series also started with an injection of adenosine, since preliminary trials had shown that the cardiovascular response to adenosine appeared attenuated compared with the results recorded at Scott Base (series 1). The adenosine injection also allowed direct comparisons with the responses induced by the subsequent injection of the A_1 receptor agonist N^6-cyclopentyladenosine (CPA, 10 nmol kg^{-1}). Because the blocking effect of the A_1-receptor antagonist 8-cyclopentyltheophylline (CPT, 50 nmol kg^{-1}) is short and the cardiovascular variables after injection stabilise quickly (Sundin and Nilsson 1996), a recovery time of only 10 min was allowed before the second and final injection of CPA and adenosine.

Drugs

N^6-cyclopentyladenosine (CPA) and 8-cyclopentyltheophylline (CPT) were obtained from Research

Biochemicals International (RBI, Natick, MA, USA). Adenosine and aminophylline were obtained from Sigma.

Data acquisition and statistics

The cardiovascular variables and the blood oxygen partial pressure were continuously recorded using a Yokogawa recorder (model 3701 LR8100), and the data were also sampled using Labview (version 4.0, National Instrument). Sampling frequency used was 30 Hz, and mean values were subsequently calculated at 15 s intervals. Values are presented as means ± S.E.M. for N animals. Branchial and systemic vascular resistances (R_{Gill} and R_{Sys}, respectively) were calculated as $(P_{VA}–P_{DA})/\dot{Q}$ and $P_{DA}/\dot{Q}$, respectively. We are aware that approximately 7–8 % of cardiac output returns directly to the heart *via* the arterio-venous pathway in rainbow trout and Atlantic cod (*Gadus morhua*) (Ishimatsu et al., 1988; Sundin and Nilsson, 1992). This will lead to a small underestimation of the calculated systemic resistance but, since we have no information about the situation in the borch, we use these simplified calculations. The error will be relatively small unless there is a massive increase in arterio-venous blood flow. The calculations also assume that venous pressure is close to zero and does not change during the different treatments. Cardiac output is given in relative terms instead of as an absolute value. In all cases, $\dot{Q}$ was expressed as kHz Doppler shift. For presentation, these values have been converted to percentage changes from pre-injection values.

The Wilcoxon signed-rank test was used to evaluate statistically significant changes ($P<0.05$) in the recorded variables at minimum and maximum values compared with pre-injection values. Comparisons between groups were made using the Mann–Whitney U-test. When more than one comparison was made, the sequentially rejective Bonferroni test (Holm, 1979) was used to minimise the possibility of discarding any true null hypothesis. The Spearman rank correlation test was used to test the correlation between resting P_{O_2} and the change in P_{O_2} induced by adenosine.

Results

Branchial vasculature

Series 1

In this group, injections of 10 nmol kg^{-1} adenosine caused a biphasic response consisting of a rapid and significant transient decrease followed by a significant and longer-lasting increase in R_{Gill}. This is reflected in the changes in P_{VA} (Fig. 1). Adenosine also significantly reduced the arterial P_{O_2} (Fig. 1). A significant correlation was found between the pre-injection arterial P_{O_2} and the final P_{O_2} (Fig. 2). Pre-treatment with the general P_1-receptor antagonist aminophylline (40 nmol kg^{-1}) abolished all these effects except the initial decrease in R_{Gill} (Fig. 1).

Series 2

The branchial effects of injected CPA (an A_1-receptor agonist, 10 nmol kg^{-1}) resembled those obtained with adenosine: a biphasic response consisting of a rapid and transient decrease followed by a significant and longer-lasting increase in R_{Gill}. However, compared with the adenosine response elicited in series 1, the increase in R_{Gill} was attenuated and there was no increase in P_{VA}, which instead showed a greater transient decrease associated with the bradycardia (Fig. 3). To compare directly the effects of adenosine and CPA, which is a very potent vasoconstrictor in trout gill vessels (Sundin and Nilsson, 1996), an injection of adenosine was also made in this series. As shown in Fig. 4, the response to adenosine in this series was essentially similar to the response to CPA. Pre-treatment with the specific A_1-receptor antagonist CPT (50 nmol kg^{-1}) abolished or significantly reduced all the branchial responses to adenosine and CPA (Figs 3, 4).

Systemic vasculature

Series 1

Adenosine caused a biphasic change in R_{Sys}, with an initial increase that preceded a long-lasting significant decrease, and at this time P_{DA} was reduced (Fig. 1). Aminophylline significantly reduced these responses, although some of the decrease in R_{Sys} and P_{DA} remained (Fig. 1).

Series 2

The changes in P_{DA} and R_{Sys} in response to adenosine were similar to those in series 1 (Fig. 4), and the responses were mimicked by CPA (Fig. 3). As with the inhibition by aminophylline in series 1, the specific A_1-receptor antagonist CPT significantly reduced the adenosine-induced systemic changes (Fig. 4) and also a significant part of the systemic changes elicited by CPA (Fig. 3).

The heart

Series 1

Adenosine produced an initial rapid and marked decrease in heart rate. This was later reversed to a small significant tachycardia. Correspondingly, there was an initial decrease in $\dot{Q}$ (Fig. 1A), but $\dot{Q}$ later increased markedly (Fig. 1). The secondary tachycardia was small, and most of the increase in $\dot{Q}$ was mediated through an increase in stroke volume. This is supported by the responses of $\dot{Q}$ and f_H to adenosine in series 2, in which $\dot{Q}$ was elevated without an increase in f_H (Fig. 4). Pre-treatment with aminophylline abolished the decrease in f_H and significantly reduced the elevation of $\dot{Q}$, although some of the increase remained (Fig. 1).

Series 2

Adenosine had comparable effects on f_H and $\dot{Q}$ in this series. As with aminophylline, CPT blocked both the decrease in f_H and a significant part of the elevation in $\dot{Q}$ (Fig. 4). CPA produced an even more marked bradycardia than adenosine and, as a result, $\dot{Q}$ decreased (Fig. 3). CPT abolished the CPA-induced reduction in f_H and consequently significantly reduced the initial decrease in $\dot{Q}$.

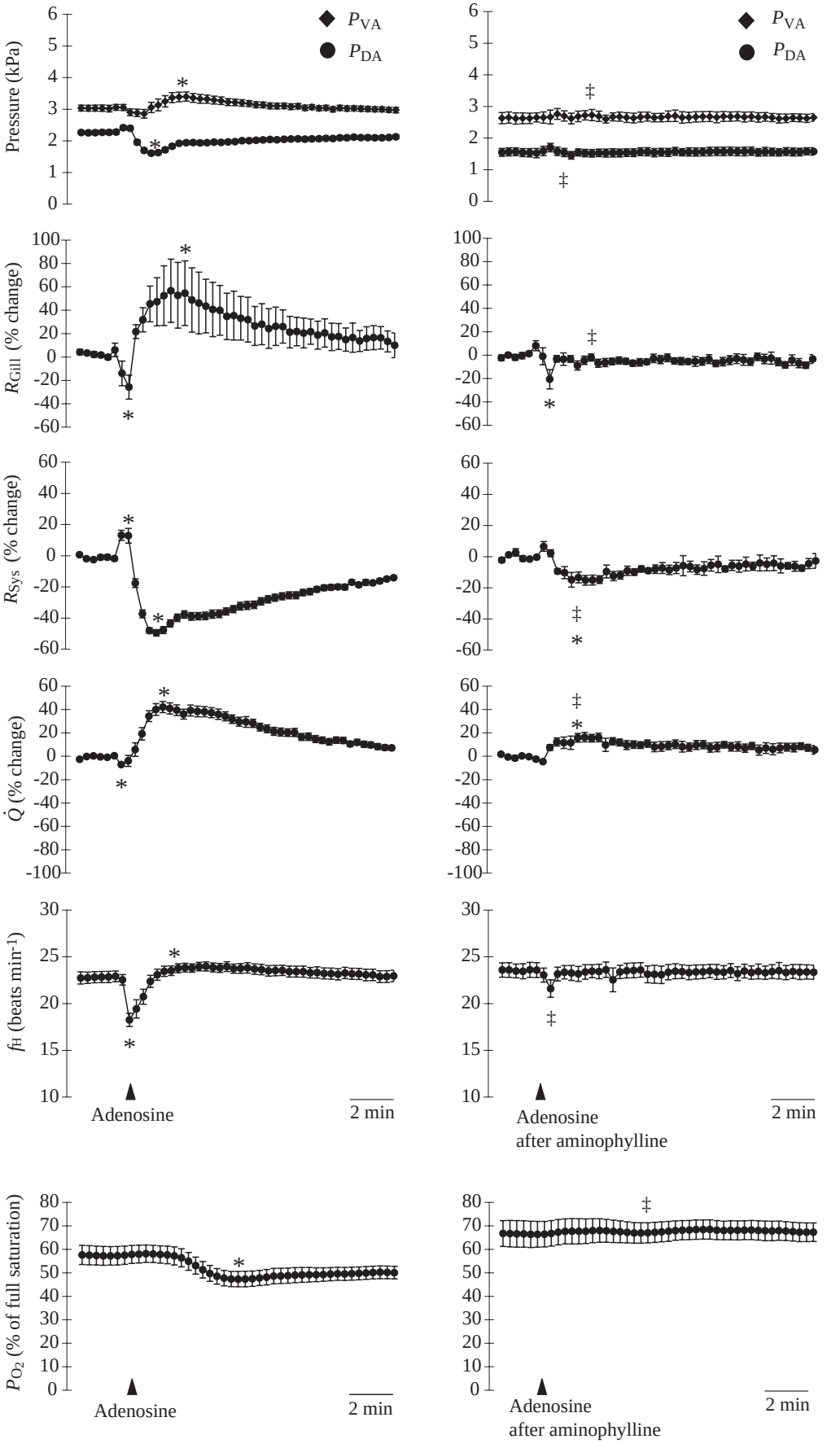

Fig. 1. A summary of the responses to intra-arterial injections of adenosine (10 nmol kg^{-1}) before (N=15) and after (N=7) treatment with aminophylline (40 nmol kg^{-1}) (a P_1-receptor antagonist) on cardiovascular variables and arterial oxygen tension. P_{VA} and P_{DA}, ventral and dorsal aortic pressure, respectively; R_{Gill}, gill resistance; R_{Sys}, systemic resistance; P_{O_2}, arterial oxygen tension, f_H, heart rate. Values are means ± S.E.M. An asterisk marks a significant difference determined at minimum and maximum recorded levels *versus* pre-injection values (P<0.05). A double dagger indicates a statistically significant difference after treatment with the antagonist (P<0.05).

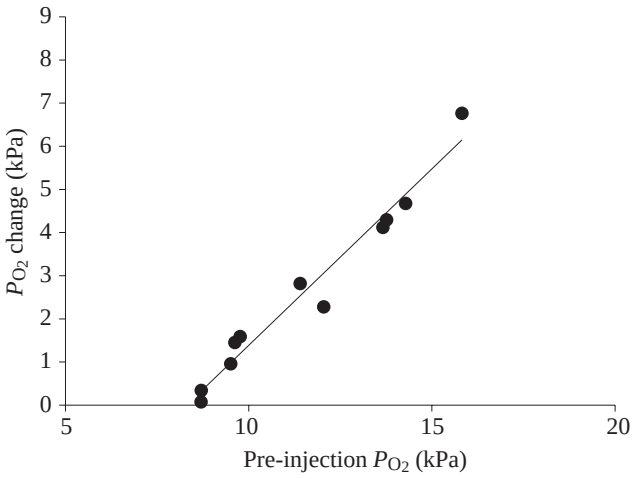

Fig. 2. A graph shows the correlation between pre-injection arterial oxygen tension (P_{O_2}) and the percentage reduction caused by injected adenosine (10 nmol kg^{-1}, N=11). The equation for the regression is y=0.82x–6.79, P<0.05, r^2=0.964.

Discussion

This is the first study to describe the effects of adenosine in an Antarctic teleost species. Basal values for heart rate and dorsal and ventral blood pressure are comparable with the values reported in earlier studies on this species (Axelsson et al., 1994). In the present study, adenosine and CPA caused a branchial vasoconstriction in borchs that could be abolished by aminophylline and CPT. Adenosine acting on P_1 receptors has been found to alter the vascular resistance of perfused fish gills (Colin and Leray, 1979; Okafor and Oduleye, 1986), and it has recently been shown that the purinergic receptor mediating the gill vasoconstriction in the rainbow trout is the A_1 receptor (Sundin and Nilsson, 1996). Judging from these studies, it seems that the A_1 receptor may be a 'general' mediator of adenosine-induced vasoconstriction in teleost gills.

Sundin and Nilsson (1996) described the locations of the adenosine-induced branchial vasoconstriction. They found that the nucleotide constricted the distal portion of all the filament blood vessels (both afferent and efferent filament arteries and arterioles). Despite an increase in cardiac output in response to adenosine, there was a redistribution of blood flow towards the base of the gill filament. This apparent reduction in the functional respiratory surface area might be expected to impair gas exchange. The extracorporeal loop used in the present study allowed measurement of arterial P_{O_2} in the borch. Adenosine did indeed reduce the arterial P_{O_2} and this effect could be blocked by aminophylline. A correlation between the pre-injection arterial P_{O_2} and the change in P_{O_2} in response to adenosine was found, with a large reduction in arterial P_{O_2} when the arterial P_{O_2} was high. In another study, Sundin et al. (1998) found a correlation between the effects of serotonin (5-HT) and arterial P_{O_2}. As in the present study, 5-HT lowered the arterial P_{O_2} with a larger reduction in P_{O_2} in animals with a higher pre-injection arterial P_{O_2}. Taken together, it is clear that the branchial resistance directly reflects the effectiveness of oxygen uptake, which in turn must be due to the size of the functional respiratory surface area.

Although 1 nmol kg^{-1} of the specific A_1-receptor agonist CPA induced a 150 % increase in the branchial resistance of the trout (Sundin and Nilsson, 1996), the borchs in the present study displayed only a modest increase in response to a 10-fold higher dose of the same agonist. We found a difference in response to adenosine between series 1 and series 2. The only apparent difference in experimental conditions between these two series was the temperature. When the serotonergic control of the branchial vasculature in borchs was studied, no difference in response to 5-HT between the 'Antarctic' and 'New Zealand' groups was found (Sundin et al., 1998). It is possible that the water temperature difference in the holding tanks between Scott Base (–1.3 °C) and Christchurch (0 °C) could influence the A_1 receptor. Supporting this idea are reports from the mammalian literature suggesting that, in contrast to A_2 receptors, the actions of A_1 receptors are temperature-dependent (Broadley et al., 1985; Stojanov and Proctor, 1990). Furthermore, it has been shown both in the hamster and in the flounder that there is a negative interaction between the adrenergic and adenosinergic receptor systems (Lennard and Huddart, 1989; Proctor and Stojanov, 1991). It is feasible that the stenothermal borchs, flown to Christchurch where they were kept at 0 °C, have a higher level of sympathetic activation and that this, in turn, may then negatively influence the adenosinergic receptors.

In mammals, adenosine-induced vasodilatation is effected by A_2 receptors (Olsson and Pearson, 1990) and vasodilatory A_2 receptors have been found in the ventral aorta of the dogfish (Evans, 1992). In borchs, injection of CPA and adenosine-induced secondary dilatation of the systemic vasculature and the responses to both agonists could be blocked by the A_1 antagonist CPT. A similar response has been observed in trout, in which a specific A_2 agonist was also used and had no effect (Sundin and Nilsson, 1996). Taken together, these findings suggest that A_1 receptors may function as systemic vasodilators in teleost fish. However, other factors could have influenced our results. One aspect to consider is the possibility of a 'passive' distension of compliant vessels in response to increased blood flow (Wood and Shelton, 1975), especially since there is a persistent adenosine-induced decrease in R_{Sys} even after treatment with aminophylline and because the magnitude of changes in R_{Sys} closely follows the magnitude of changes in $\dot{Q}$. Also intriguing is the initial and transient adenosine- and CPA-induced increase in R_{Sys} that can be reduced by both antagonists. Since this response also reflects the initial changes in $\dot{Q}$, it supports the idea that a portion of the resistance changes in the systemic vasculature might be due to the inherent compliance of the vessels. In our calculation of R_{sys}, we assume that a constant proportion of the cardiac output flows through the systemic vessels. As noted above, adenosine diverts branchial blood to the arterio-venous pathway (Sundin and Nilsson, 1996). Although this pathway accounts for only a relatively small 7 % of the total branchial flow in the rainbow trout and 8 % the Atlantic cod (Ishimatsu et al., 1988; Sundin

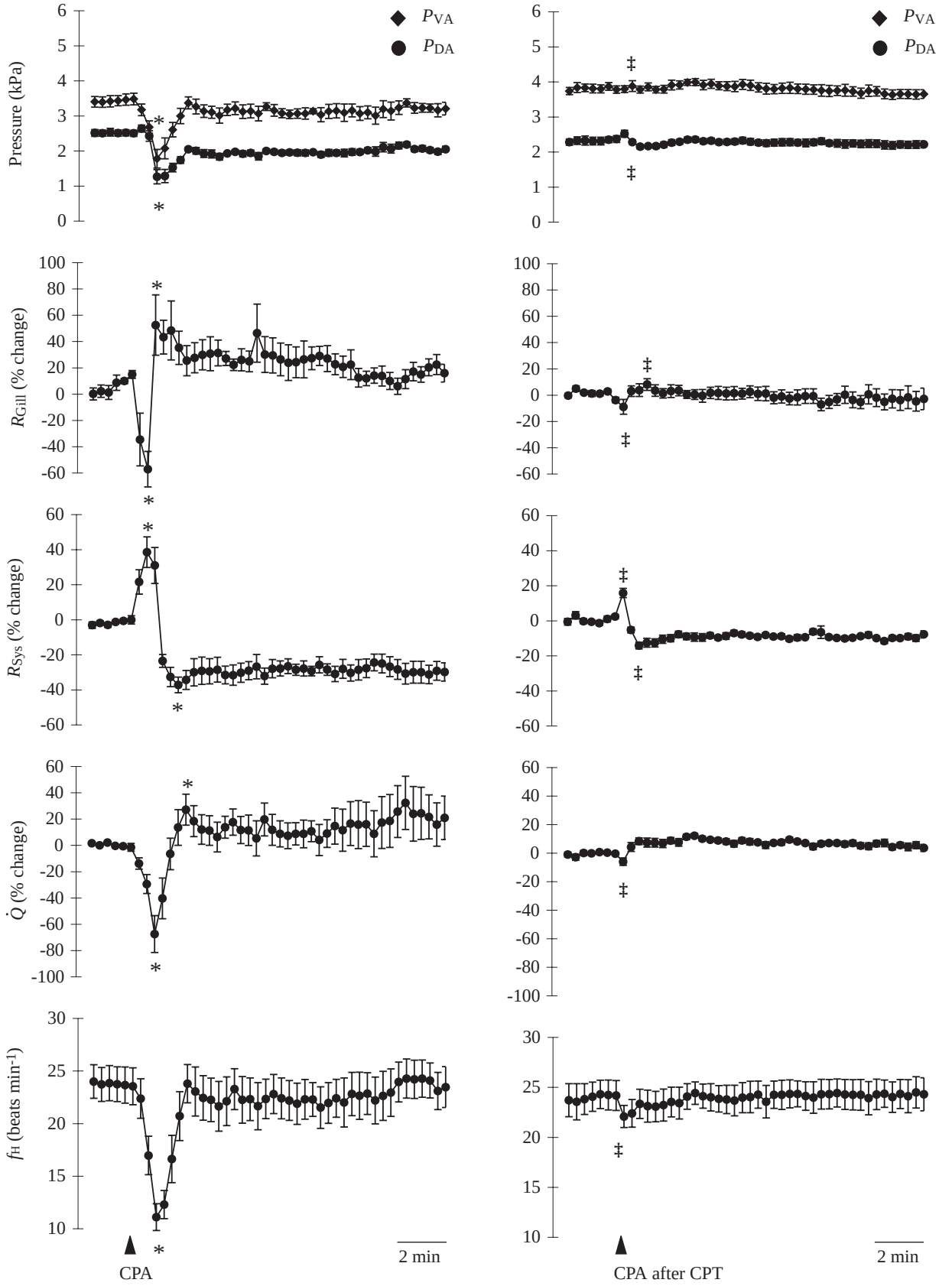

Fig. 3. A summary of the cardiovascular responses to intra-arterial injections of N^6-cyclopentyladenosine (CPA, an A_1-receptor agonist; 10 nmol kg^{-1}) before (N=7) and after (N=7) treatment with 8-cyclopentyltheopylline (CPT, an A_1-receptor antagonist; 50 nmol kg^{-1}). See Fig. 1 for further details.

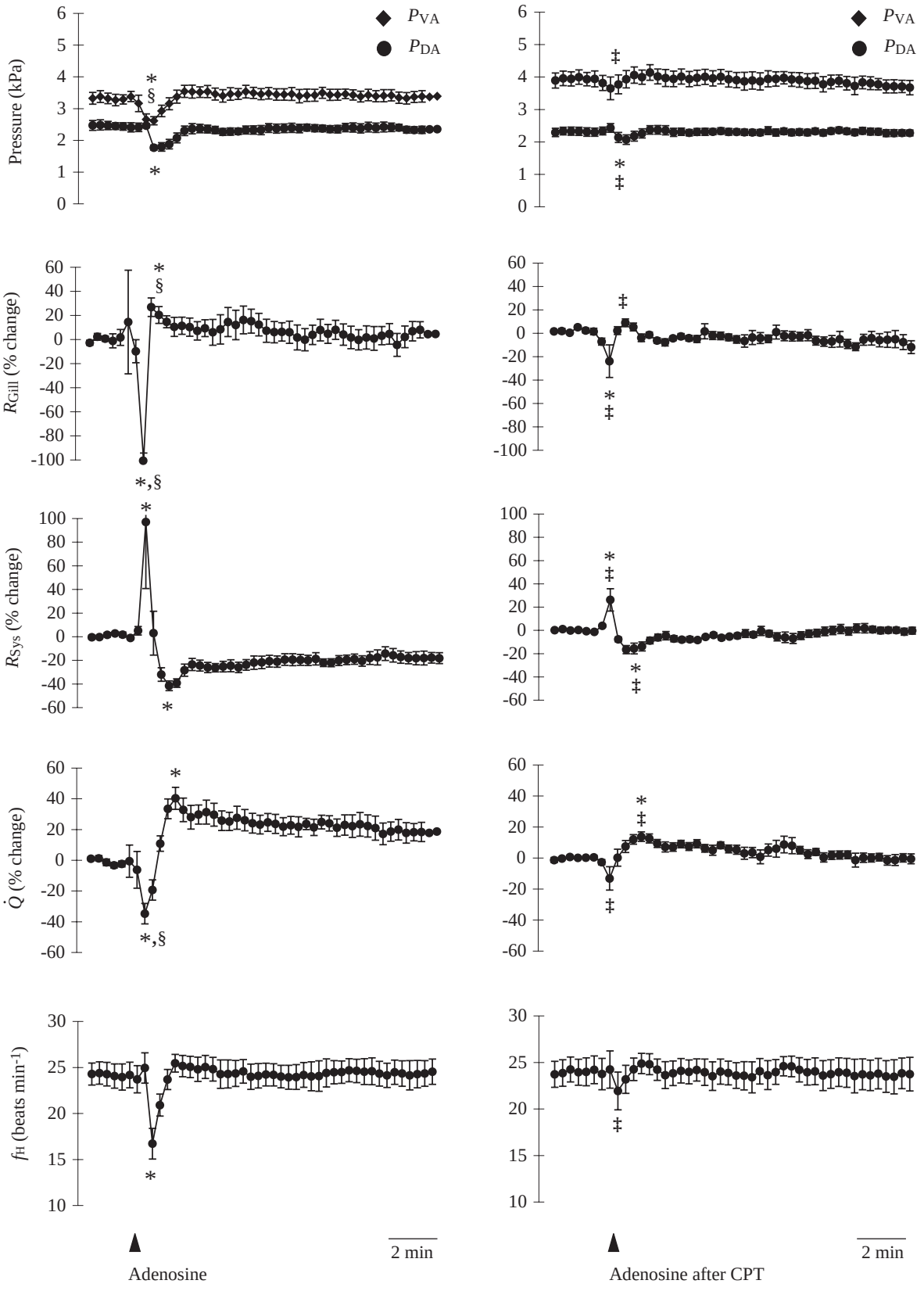

Fig. 4. A summary of the cardiovascular responses to intra-arterial injections of adenosine (10 nmol kg^{-1}) before (N=7) and after (N=7) treatment with 8-cyclopentyltheopylline (CPT, an A_1-receptor antagonist; 50 nmol kg^{-1}). Note the attenuated response compared with Fig. 1. § indicates a statistically significant difference (P<0.05) between adenosine treatments in borchs at Christchurch and Scott Base. See Fig. 1 for further details.

and Nilsson, 1992), this and other potential changes in flow patterns might divert blood away from the systemic circulation and so result in an underestimation of R_{sys}. R_{sys} would also be underestimated if venous pressure rose. Further, flow-induced vasodilatation could result from increased blood flow releasing vasoactive substances within endothelial cells (Burnstock, 1993).

Adenosine and its analogues have been found to have a negative chronotropic effect on the heart in various animal groups (see Olsson and Pearson, 1990; Burnstock, 1996). Recently, the first *in vivo* responses to adenosine by the fish heart have been reported: trout displayed a slowing of their heart rate. However, because P_{VA} increased simultaneously, there is a possibility that the decreased f_H was a baroreceptor reflex (Sundin and Nilsson, 1996). In series 2 in the present study, the bradycardia elicited by adenosine and particularly by the A_1-receptor agonist CPA occurred despite the absence of raised blood pressures, eliminating the possibility of a barostatic reflex. Instead, this heart rate response must have been caused by adenosinergic mechanisms elicited by the A_1 receptor. There are several possible ways in which A_1 receptors might cause a negative chronotropic response: (1) through direct actions on cardiac myocytes and endothelial cells; (2) by inhibiting the release of adrenaline from adrenergic nerve terminals in the heart; (3) by activating arterial chemoreceptors; and (4) through central effects within the nucleus tractus solitarius (Olsson and Pearson, 1990; Pelleg et al., 1996). In relation to the fourth possibility, A_1 receptors have been found in the fish brain (Burnstock, 1996).

Initially $\dot{Q}$ fell with f_H in response to adenosine. While f_H returned to resting levels (slightly above resting levels in series 1), $\dot{Q}$ increased markedly. The elevation in $\dot{Q}$ must therefore have been due to an increase in the stroke volume. This could have occurred *via* a positive inotropic effect on the heart or as a consequence of raised filling pressures, because venous return is the major determinant of cardiac output in fish (Olson, 1997). The same positive effect of adenosine on the stroke volume of live fish has been observed in trout (Sundin and Nilsson, 1996). In other reports, adenosine has been shown to decrease atrial contractility but to have no effect on ventricular muscle (Burnstock and Meghji, 1981; Cohen et al., 1981; Meghji and Burnstock, 1984; Rotmensch et al., 1981). In fact, there is some uncertainty concerning the extent to which some of the *in vitro* observations in mammals translate into a physiological role for adenosine as a regulator of cardiac performance *in vivo* (Olsson and Pearson, 1990). In fish, filling of the ventricle depends almost exclusively on atrial contraction (Johansen, 1971). It is therefore impossible *in vivo* to determine whether the agonists affected both the atrium and the ventricle. Positive effects of adenosine have been demonstrated on both atrial and ventricular muscle strips of the flounder (*Platichthys flesus*) (Lennard and Huddart, 1989) and on the ventricle of the frog (*Xenopus laevis*) (Meghji and Burnstock, 1983).

In conclusion, we have shown that adenosine is a potent cardiovascular regulator in borchs, suggesting an evolutionarily conserved cardiovascular control function in teleosts. A_1 receptors mediate excitatory and possibly also inhibitory effects on the vasculature at different locations. The adenosine-induced branchial vasoconstriction decreased the arterial P_{O_2}. Adenosine also induced a bradycardia and increased stroke volume. These responses may also be elicited by A_1-receptor-activated mechanisms.

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References

Axelsson, M., Davison, W., Forster, M. E. and Nilsson, S. (1994). Blood pressure control in the Antarctic fish *Pagothenia borchgrevinki*. *J. Exp. Biol.* **190**, 265–279.

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.

Broadley, K. J., Broome, S. and Paton, D. M. (1985). Hypothermia-induced supersensitivity to adenosine for responses mediated *via* A_1-receptors but not A_2-receptors. *Br. J. Pharmac.* **84**, 407–416.

Burnstock, G. (1993). Integration of factors controlling vascular tone. *Anesthesiology* **79**, 1368–1380.

Burnstock, G. (1996). Purinoreceptors: ontogeny and phylogeny. *Drug Dev. Res.* **39**, 204–242.

Burnstock, G. and Meghji, P. (1981). Distribution of P1- and P2-purinoceptors in the guinea-pig and frog heart. *Br. J. Pharmac.* **73**, 879–885.

Cohen, S., Rotmensch, H. H., Rubinstein, R. and Lass, Y. (1981). Lack of uptake or degradation of adenosine in the termination of its action in the beating carp atrium. *Biochem. Pharmac.* **30**, 890–893.

Colin, D. A. and Leray, C. (1979). Interaction of adenosine and its phosphorylated derivatives with putative purinergic receptors in the gill vascular bed of rainbow trout. *Pflügers Arch.* **383**, 35–40.

Colin, D. A. and Leray, C. (1981). Vasoactivities of adenosine analogs in trout gill (*Salmo gairdneri* R.). *Biochem. Pharmac.* **30**, 2971–2978.

Davison, W., Axelsson, M., Nilsson, S. and Forster, M. E. (1997). Cardiovascular control in Antarctic notothenioid fishes. *Comp. Biochem. Physiol.* **118**A, 1001–1008.

Evans, D. H. (1992). Evidence for the presence of A_1 and A_2 adenosine receptors in the ventral aorta of the dogfish shark, *Squalus acanthias*. *J. Comp. Physiol.* **162**, 179–183.

Forster, M. E., Forster, A. H. and Davison, W. (1998). Effects of serotonin, adrenaline and other vasoactive drugs on the branchial blood vessels of the Antarctic fish *Pagothenia borchgrevinki*. *Fish Physiol. Biochem.* **19**, 103–109.

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

Ishimatsu, A., Iwama, G. K. and Heisler, N. (1988). *In vivo* analysis of partitioning of cardiac output between systemic and central venous sinus circuits in rainbow trout: a new approach using chronic cannulation of the branchial vein. *J. Exp. Biol.* **137**, 75–88.

Johansen, K. (1971). Comparative physiology: Gas exchange and circulation in fishes. *Annu. Rev. Physiol.* **33**, 569–612.

Lennard, R. and Huddart, H. (1989). Purinergic modulation of cardiac activity in the flounder during hypoxic stress. *J. Comp. Physiol.* **159**, 105–114.

Leray, C., Raffin, J. P. and Winninger, C. (1978). Aspects of purine metabolism in the gill epithelium of rainbow trout, *Salmo gairdneri* Richardson. *Comp. Biochem. Physiol.* **62**, 31–40.

Meghji, P. and Burnstock, G. (1983). An unusual excitatory action of adenosine on the ventricular muscle of the south African clawed toad (*Xenopus laevis*). *Eur. J. Pharmac.* **89**, 251–258.

Meghji, P. and Burnstock, G. (1984). The effects of adenyl compounds on the heart of the dogfish, *Scyliorhinus canicula*. *Comp. Biochem. Physiol.* **77**C, 295–300.

Okafor, M. C. J. and Oduleye, S. O. (1986). Hemodynamic effects of purinergic receptor stimulation in isolated fish gills. *Acta Physiol. Hung.* **67**, 45–51.

Olson, K. R. (1997). The cardiovascular system. In *The Physiology of Fishes*, 2nd edition (ed. D. H. Evans), pp. 129–154. Boca Raton, FL: CRC Press.

Olsson, R. A. and Pearson, J. D. (1990). Cardiovascular purinoceptors. *Physiol. Rev.* **70**, 761–845.

Pelleg, A., Katchanov, G. and Xu, J. (1996). Purinergic modulation of neural control of cardiac function. *J. Autonom. Pharmac.* **16**, 401–405.

Proctor, K. G. and Stojanov, I. (1991). Direct vasoconstriction evoked by A_1-adenosine receptor stimulation in the cutaneous microcircualtion. *Circulation Res.* **68**, 683–688.

Rotmensch, H. H., Cohen, S., Rubinstein, R. and Lass, Y. (1981). Effects of adenosine in the isolated carp atrium. *Isr. J. Med. Sci.* **17**, 393 (abstract).

Shyrock, J. C. and Belardinelli, L. (1997). Adenosine and adenosine receptors in the cardiovascular system: Biochemistry, physiology and pharmacology. *Am. J. Cardiol.* **79**, 2–10.

Siebenaller, J. F. and Murray, T. F. (1986). Phylogenetic distribution of ^{3}H-cyclohexyladenosine binding sites in nervous tissue. *Biochem. Biophys. Res. Commun.* **137**, 182–189.

Silinsky, E. M. and Redman, R. S. (1996). Synchronous release of ATP and neurotransmitter within milliseconds of a motor nerve impulses in the frog. *J. Physiol., Lond.* **492**, 815–822.

Stojanov, I. and Proctor, K. G. (1990). Temperature-sensitive adenosine-mediated vasoconstriction in the skin microcirculation. *J. Pharmac. Exp. Ther*. **253**, 1083–1089.

Sundin, L., Davison, W., Forster, M. E. and Axelsson, M. (1998). A role of 5-HT_2 receptors in the gill vasculature of the Antarctic fish *Pagothenia borchgrvinki*. *J. Exp. Biol*. **201**, 2129–2138.

Sundin, L. and Nilsson, G. E. (1996). Branchial and systemic roles of adenosine receptors in rainbow trout: an *in vivo* microscopy study. *Am. J. Physiol*. **40**, R661–R669.

Sundin, L. and Nilsson, S. (1992). Arterio-venous branchial blood flow in the Atlantic cod *Gadus morhua*. *J. Exp. Biol.* **165**, 73–84.

Wood, C. M. and Shelton, G. (1975). Physical and adrenergic factors affecting systemic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. Exp. Biol.* **63**, 505–523.