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A ROLE OF 5-HT$_2$ RECEPTORS IN THE GILL VASCULATURE OF THE ANTARCTIC FISH *PAGOTHENIA BORCHGREVINKI*

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Summary

This study was conducted to describe the cardiovascular responses to intra-arterial injections of serotonin in the Antarctic fish *Pagothenia borchgrevinki* and to elucidate the underlying mechanisms. Immunohistochemistry was used to localise serotonin-containing cells within the gills. 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 in combination with Doppler flow measurement. An extracorporeal loop with an in-line oxygen electrode allowed continuous measurements of arterial oxygen pressure Pa_{O_2}. Pre-branchial injection of serotonin (5-hydroxytryptamine, 5-HT) or the 5-HT$_2$ receptor agonist α-methylserotonin increased the branchial vascular resistance and ventral aortic pressure, while the 5-HT$_1$ receptor agonist piperazine was without effect. The branchial vasoconstriction produced by serotonin injection was completely blocked by the 5-HT$_1$/5-HT$_2$ receptor antagonist methysergide and the branchial vasoconstriction produced by α-methylserotonin injection was completely blocked by the specific 5-HT$_2$ receptor antagonist LY53857. The results suggest that the 5-HT$_2$ receptor alone mediates the branchial vasoconstriction. Serotonin also mediated a methysergide-sensitive reduction in Pa_{O_2}, the reduction being greatest when the pre-injection Pa_{O_2} value was high. 5-HT-immunoreactive cells and nerve fibres were present within the gill tissues. All the 5-HT-immunoreactive cells were located on the efferent side of the filaments, but 5-HT-immunoreactive nerve fibres were found lining both of the branchial arteries. Our findings demonstrate a potential serotonergic control system for the gills in *Pagothenia borchgrevinki*. In contrast to its effects on the branchial vasculature, serotonin produced a methysergide-insensitive decrease in the systemic vascular resistance. However, neither the specific 5-HT$_1$ nor 5-HT$_2$ receptor agonists produced a decrease in the resistance of the systemic vasculature. The nature of the serotonergic receptor(s) inducing vasodilation in teleost fish is uncertain.

Key words: teleost, 5-hydroxytryptamine, circulation, blood vessel, gill, oxygen tension, *Pagothenia borchgrevinki*.

Introduction

The presence of both cholinergic and adrenergic control mechanisms in the branchial vasculature of the notothenioid Antarctic fish *Pagothenia borchgrevinki* ('borch') has been demonstrated (Axelsson *et al.* 1994), with α-adrenergic vasoconstrictor activity predominating over β-adrenergic vasodilatation. This prevalence of α-adrenoceptors in the branchial vasculature in borch has also been confirmed by experiments on isolated perfused gill arches (Forster *et al.* 1998). The dominance of α-adrenoceptor mechanisms over β-adrenoceptor effects on the cardiovascular system is interesting because, in teleost fish from temperate latitudes, β-adrenoceptor-mediated responses are dominant in summertime, while α-adrenoceptor-mediated responses are relatively more important during the winter (Pärt *et al.* 1982; Randall and Perry, 1992).

In addition, results from a recent study by Forster *et al.* (1998) show that serotonin (5-hydroxytryptamine, 5-HT) is more potent than both adrenaline and acetylcholine in constricting the branchial circulation in the borch. Serotonergic storage sites have been identified within the gills of every fish species so far studied (e.g. Dunel-Erb *et al.* 1982, 1989; Bailly *et al.* 1989, 1992; Zaccone *et al.* 1992; Sundin, 1996). The monoamine-induced branchial vasoconstriction can be blocked by the 5-HT$_1$/5-HT$_2$ receptor antagonist methysergide (Atlantic cod *Gadus morhua*, Östlund and Fänge, 1962; rainbow trout *Oncorhynchus mykiss*, Katchen *et al.* 1976; Fritsche *et al.* 1992; Sundin *et al.* 1995; eel *Anguilla anguilla*, Janvier *et al.* 1996*b*). Therefore, it is possible that serotonergic mechanisms are a component of the excitatory non-adrenergic and non-cholinergic branchial vascular control previously suggested by Pettersson and Nilsson (1979).

Measurements of oxygen partial pressure (P_{O_2}) in the blood have shown that injection of serotonin is accompanied by a decrease in arterial P_{O_2} (Pa_{O_2}) in rainbow trout *Oncorhynchus*

mykiss (Thomas *et al.* 1979; Fritsche *et al.* 1992). This is presumably due to the observed constriction of filamental arteries and arterioles induced by serotonin in the same species (Sundin *et al.* 1995). Preliminary results indicate that serotonin reduces the P_{aO_2} in borch as well (Forster *et al.* 1998). Nevertheless, the physiological significance of the serotonergic haemodynamic effects and the impact on gas exchange are unclear (Fritsche *et al.* 1992; Sundin *et al.* 1995).

It has been shown that branchial $5HT_1/5HT_2$ receptors are involved in the 5-HT-induced increase in branchial resistance in teleosts; however, no attempts have been made to distinguish further the receptor mechanism involved. Furthermore, the *in vitro* study by Forster *et al.* (1998) on isolated borch gill arches and gill vessels indicates a branchial vasculature very sensitive to serotonin. No *in vivo* studies have been performed to elucidate the functional significance of the serotonergic control mechanisms, and there is also a lack of information regarding the presence and localization of serotonin in the branchial vasculature of borch. The present study was undertaken to investigate the branchial and systemic responses to intravascularly injected serotonin in the Antarctic fish *P. borchgrevinki*, to identify the receptor type involved and to localise branchial storage sites for serotonin.

Materials and methods

Animals

The red-blooded Antarctic notothenioid fish *Pagothenia borchgrevinki* (Boulenger) (commonly called 'borch') was caught by line and hook in McMurdo Sound, Antarctica, and transported back to Scott Base (New Zealand Antarctic Programme) where they were kept in plastic tanks at −1.3 to −0.8 °C. Animals were also flown to Christchurch, New Zealand, where part of this study was undertaken. In Christchurch, the animals were held in plastic tanks in a closed-circuit sea water aquarium system at −0.5 °C.

Surgery

Twenty-one fish with body mass of 114.3±5.8 g (mean ± S.E.M.) were used in these studies. Prior to surgery, animals were given at least 48 h to recover from the effects of capture, and they were subsequently allowed 24 h to recover from surgery before measurements were started.

The surgery, including cannulation of the afferent and efferent branchial arteries and placement of a Doppler flow probe around the ventral aorta, was as described by Axelsson *et al.* (1994), as was the equipment used to record pressures and flows. An extracorporeal loop was established by pumping dorsal aortic blood *via* the efferent branchial cannula into the afferent branchial cannula *via* a flow-through oxygen electrode, allowing continuous recording of dorsal aortic oxygen tension. Blood was pumped around the extracorporeal loop using a Minipuls 3 pump (Gilson, Villiers-le-Bel, France). The volume of blood in the loop was approximately 180 µl, and the total circulation time was approximately 2 min. The Clark-type flow-through electrode (type 16-73) and meter (type OM-4) used to measure arterial oxygen partial pressure were manufactured by Microelectrodes Inc. (Londonderry, NH, USA). The oxygen electrode was submerged in the sea water at the same temperature (−0.5 to 0 °C) as the fish and was zeroed using sodium sulphite solution in sodium borate. The electrode was calibrated using air-saturated sodium chloride solution (0.9 %) and proved to be stable during the experimental period; it had a 95 % response time of less than 45 s at the experimental temperature. When the data were analysed, allowance was made both for the time taken for sampled blood to reach the oxygen electrode and for the response time of the electrode itself.

Experimental protocol

Individual fish were kept in the experimental chambers (approximately 170 mm×250 mm and 120 mm deep) for at least 24 h postsurgery to allow sufficient time for recovery from implantation of cannulae and the flow probe and to let the animals become accustomed to the environment. Since it is impossible, with the present arrangement, to record cardiovascular variables and oxygen partial pressures simultaneously, the animals were first connected for recordings of ventral (P_{VA}) and dorsal (P_{DA}) pressures, heart rate (f_H) and cardiac output (Q). This allowed us to check whether the ventral and dorsal aortic cannulae were patent and that the animals had recovered sufficiently from surgery. Fish without apparent beat-to-beat heart rate variation were not used since a reduced cholinergic tone on the heart, leading to a low beat-to-beat variation in heart rate, is indicative of stress.

In preliminary trials, we tested for suitable dosages of agonist drugs. The doses of agonists chosen gave clear and consistent submaximal effects, and the selected doses of antagonists were sufficient to cause blockage.

Series 1

This part of the study was conducted at Scott Base, Antarctica. In this group, 0.1 nmol kg^{-1} (100 µl kg^{-1}, 10^{-5} mol l^{-1}) serotonin was administered twice before and twice after treatment with methysergide (1 mg kg^{-1}, 2 µmol kg^{-1}, a non-selective $5\text{-}HT_1/5\text{-}HT_2$ receptor antagonist).

During the first injection, effects on cardiovascular parameters were measured; during the second, P_{aO_2} was measured. At the beginning of each experiment, resting values of the measured variables (Q, P_{VA}, P_{DA}, f_H, P_{aO_2}) were recorded, and after methysergide treatment the animals were left to recover for at least 30 min or until the cardiovascular variables had stabilised to pre-treatment values.

Series 2

This part of the study was conducted in Christchurch, New Zealand. To evaluate the serotonergic receptors involved in the cardiovascular responses obtained in series 1, specific agonists and antagonists for $5\text{-}HT_1$ and $5\text{-}HT_2$ receptors were administered sequentially. When the cardiovascular variables were stable, α-methylserotonin (a $5\text{-}HT_2$ receptor agonist;

0.1 nmol kg^{-1}, 100 µl kg^{-1}, 10^{-5} mol l^{-1}) was injected and the effects recorded. After all the recorded variables had returned to resting values, piperazine (0.1 nmol kg^{-1}, 100 µl kg^{-1}, 10^{-5} mol l^{-1}), a 5-HT$_1$ receptor agonist, was administered and the effect was recorded. Then the fish were pre-treated with the 5-HT$_2$ receptor antagonist LY53857 (0.5 mg kg^{-1}, 1 µmol kg^{-1}) and, when the cardiovascular parameters were stable, a final injection of α-methylserotonin was given.

Drugs

The following drugs were used in this study: serotonin hydrochloride (Sigma), chlorophenyl piperazine (Sigma), α-methylserotonin maleate (Research Biochemicals International, RBI), LY53857 maleate (RBI) and methysergide dimaleate (Sandoz). All drug injections were made into the efferent arterial cannula.

Data acquisition and statistics

The cardiovascular variables and $P_{a_{O_2}}$ were recorded continuously using a Yokogawa recorder (model 3701 LR8100), and the data were also sampled using LabView (version 4.0, National Instruments). Sampling frequency was 30 Hz, and mean values were subsequently created at 15 s intervals. Data are presented as means ± S.E.M. for N animals. Branchial and systemic vascular resistance (R_{Gill} and R_{Sys}, respectively) were calculated as $(P_{VA}-P_{DA})/Q$ and P_{DA}/Q, respectively, assuming that the entire cardiac output passed through the systemic vessels and that venous pressure was close to zero and did not change substantially during the experiments. Cardiac output was measured using the pulsed Doppler technique. Since no calibration of the flow signal was performed, the resistance changes described above are expressed as a percentage change from the value for the control period.

Evaluation of statistically significant changes (P<0.05) in the recorded variables was made using the Wilcoxon signed-rank test. To test the correlation between resting $P_{a_{O_2}}$ levels and the change in $P_{a_{O_2}}$ induced by serotonin (see Fig. 2), a Spearman rank correlation test was used.

Immunohistochemistry

The histochemical procedure followed that outlined in Karila *et al.* (1995). Four whole gill arches from either the left or the right side were excised from four fish killed by a sharp blow to the head. The gill arches were fixed in 15 % saturated picric acid and 2 % formaldehyde in 0.1 mol l^{-1} phosphate-buffered saline (PBS, pH 7.3) for 24 h at 4 °C. They were then rinsed and dehydrated in an ethanol series, treated with xylene for 30 min, rehydrated in ethanol and stored in PBS containing 30 % sucrose as a cryoprotectant until cut sections containing 3–4 filament pairs from the middle portion of each gill arch were quick-frozen in isopentane (2-methylbutane), cooled in liquid nitrogen, cut into 10 µm thick sections using a cryostat and dried onto Vectabond- (Vector) coated slides. Since the bone of borch is less calcified than that of most other teleosts (Eastman and DeVries, 1981, 1982), it was possible to cut through the whole gill arch to obtain sections containing the branchial arteries. The sections were incubated in a moist chamber at room temperature (22–24 °C) with the primary antiserum (NA-08-324, dilution 1:500, CRB, Cambridge, UK, host rabbit) for single staining for 24 h. The preparations were rinsed three times in hypertonic PBS plus 2.0 % NaCl for 5 min and incubated with secondary antibodies (DaR-DTAF, dilution 1:100, Jackson Immunores., USA, host donkey) for 1–2 h. The secondary antibodies were conjugated to dichlorotriazinyl amino fluorescein (DTAF). They were diluted in hypertonic PBS to reduce non-specific binding to the tissue sections (Grube, 1980). Preparations were viewed with an Olympus Vanox fluorescence microscope and photographed with a Leitz Orthomat camera using Kodak T-MAX 400 film rated at 1000 ASA.

To control for the specificity of the secondary antibodies, sections were either pre-incubated with normal donkey serum (1:10), or the primary antiserum was omitted from the staining procedure. We use the term 'immunoreactive' (abbreviated as 'IR') when referring to the immunohistochemical localization of serotonin.

Results

Cardiovascular experiments

Cardiac output measurements were used only to calculate branchial and systemic resistance; since none of the agonists or antagonists used had any significant effect on cardiac output, values of this variable are not included below.

Series 1

Injections of serotonin caused a significant increase in R_{Gill}, which resulted in a significant elevation of P_{VA} (Fig. 1A). The vascular events were accompanied by a reduction in arterial $P_{a_{O_2}}$ (Fig. 1B). Pre-treatment with the 5HT$_1$/5HT$_2$ receptor antagonist methysergide abolished these effects (Fig. 1). In contrast, P_{DA} decreased significantly as a result of the reduced R_{Sys} (Fig. 1A) and pre-treatment with methysergide did not alter this systemic effect of serotonin (Fig. 1B).

The magnitude of the reduction in $P_{a_{O_2}}$ after serotonin injection was significantly correlated with resting $P_{a_{O_2}}$ levels (Fig. 2).

Series 2

The branchial effects of injected α-methylserotonin (a 5-HT$_2$ receptor agonist) resembled those obtained with serotonin: a significant increase in R_{Gill} and P_{VA} (Fig. 3). Pre-treatment with a specific 5-HT$_2$ receptor antagonist (LY53857) blocked the increase in R_{Gill} and R_{Sys} (Fig. 3). In contrast to the effects of serotonin, α-methylserotonin did not significantly reduce R_{Sys} (Fig. 3). Piperazine (a 5-HT$_1$ receptor agonist) lacked effect on any of the cardiovascular variables measured (Fig. 4).

Immunohistochemistry

Two morphologically different types of 5-HT-

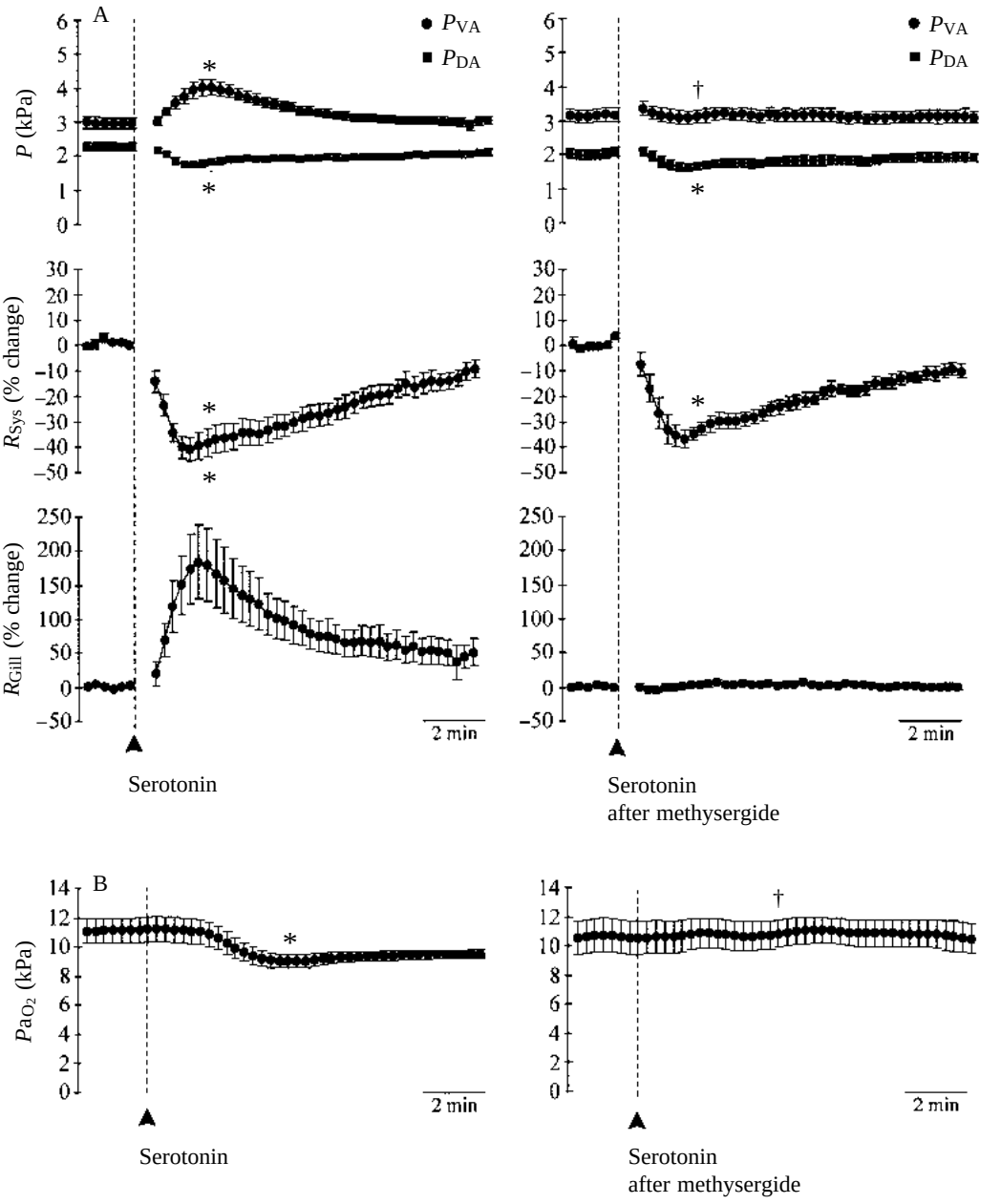

Fig. 1. The effects of intra-arterial injections of serotonin (0.1 nmol kg^{-1}) before (N=11, left panel) and after (N=8, right panel) treatment with 1 mg kg^{-1} methysergide (a 5-HT$_1$/5-HT$_2$ receptor antagonist) on cardiovascular parameters (A) and arterial oxygen tension (B). P, pressure; P_{VA} and P_{DA}, ventral and dorsal aortic pressure, respectively; R_{Gill}, gill resistance; R_{Sys}, systemic resistance; Pa_{O_2}, arterial oxygen tension. Values are means ± S.E.M. An asterisk indicates a significant difference between values determined 2 min (A) and 4 min (B) min after injection *versus* pre-injection values. A dagger indicates a statistical difference between the effects of the agonist before (left panel) and after (right panel) methysergide treatment. Injections are indicated by upward-pointing arrowheads. The discontinuous traces in A are due to injection artefacts in the dorsal aortic pressure trace affecting the resistance calculations.

immunoreactive (5-HT-IR) cell could be identified on the efferent side of the filament on all four gill arches. While round cells without short processes or with only a few short processes were concentrated in the distal third of the filament, bipolar neurones with long processes ran parallel to the filament along the efferent side (Figs 5A, 6A). Varicose nerve fibres were also seen along the filaments (Fig. 6B). At the individual filamental tips, the round cells formed discrete groups that were invariably located outside the efferent filamental artery close to the external medium (Fig. 5B). The cells in the afferent filamental artery in Fig. 5B are autofluorescent red blood cells and are not 5-HT-immunoreactive. These cells resemble the neuroepithelial cells (NECs) described by previous authors in other species (e.g. Dunel-Erb *et al.* 1982; Laurent, 1984; Bailly *et al.* 1992). In addition, there was a rich 5-HT-IR innervation

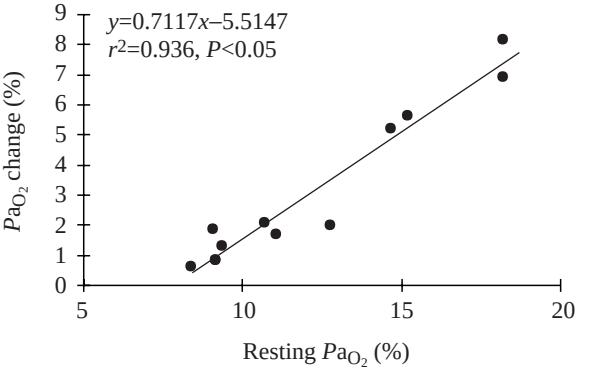

Fig. 2. The correlation between resting arterial oxygen tension (Pa_{O_2}) and the reduction in Pa_{O_2} caused by injection of serotonin (0.1 nmol kg^{-1}, N=11).

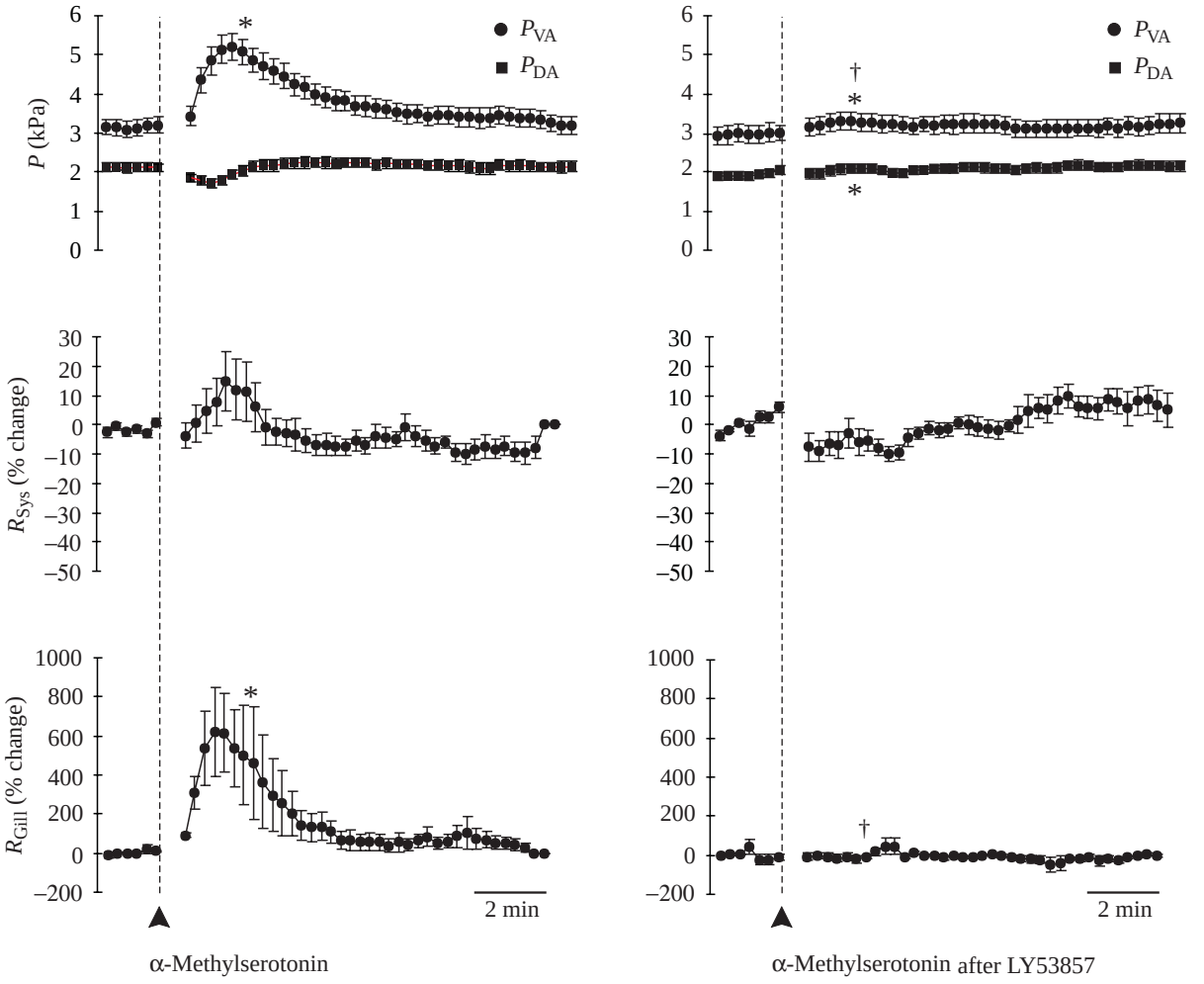

Fig. 3. The effects of intra-arterial injections of α-methylserotonin (a 5-HT_2 receptor agonist, 0.1 nmol kg^{-1}) before (N=10, left panel) and after (N=10, right panel) treatment with LY53857 (a 5-HT_2 receptor antagonist) on cardiovascular variables. Abbreviations as in Fig. 1. Values are mean ± S.E.M. An asterisk indicates a significant difference between values determined 2 min after injection and pre-injection values. A dagger indicates a significant difference between the effect of α-methylserotonin before and after treatment with LY53857. Injections are indicated by the upward-pointing arrowheads. The discontinuous traces in A are due to injection artefacts in the dorsal aortic pressure trace affecting the resistance calculations.

of the basal parts of the efferent filamental arteries of the sphincter area (Fig. 7A). No 5-HT-IR cells were distinguished along the afferent filamental arteries (non-specifically stained red blood cells can be seen in Fig. 5B in the afferent filamental artery). However, 5-HT-IR fibres were found innervating the outer walls of both the afferent and efferent branchial arteries (Fig. 7B). Peculiar 5-HT-IR cells were commonly observed on the opposite side of the gill arch towards the buccal cavity. They formed groups of at least two cells (sometimes more cells could be seen) oriented like flower-cups at the ends of branch-like structures in the gill rakers (Fig. 8).

Discussion

The present investigation demonstrates clearly that the branchial vasoconstriction caused by serotonin in *P. borchgrevinki* is mediated by a 5-HT_2 receptor, since the serotonin-activated increase in R_{Gill} and P_{VA} (Fig. 1A) was mimicked by α-methylserotonin (a specific 5-HT_2 receptor agonist) (Fig. 3) but not by piperazine (a specific 5-HT_1 receptor agonist) (Fig. 4). In addition, the constriction of the gill vessels by α-methylserotonin was effectively blocked by LY53857 (Fig. 3), a specific 5-HT_2 receptor antagonist, which further supports this conclusion. Because the vascular responses to serotonin, which are suppressed by methysergide (a general 5-HT_1/5-HT_2 receptor antagonist) (Fig. 1), are similar to those obtained in all other species investigated (Atlantic cod *Gadus morhua*, Östlund and Fänge, 1962; rainbow trout *Oncorhynchus mykiss*, Katchen *et al.* 1976; Fritsche *et al.* 1992; Sundin *et al.* 1995; eel *Anguilla anguilla*, Janvier *et al.* 1996*b*), it seems very likely that the 5-HT_2 receptor is a 'general' mediator of serotonergic vasoconstriction in the teleost branchial vasculature. It is also interesting to note the difference in the concentration of

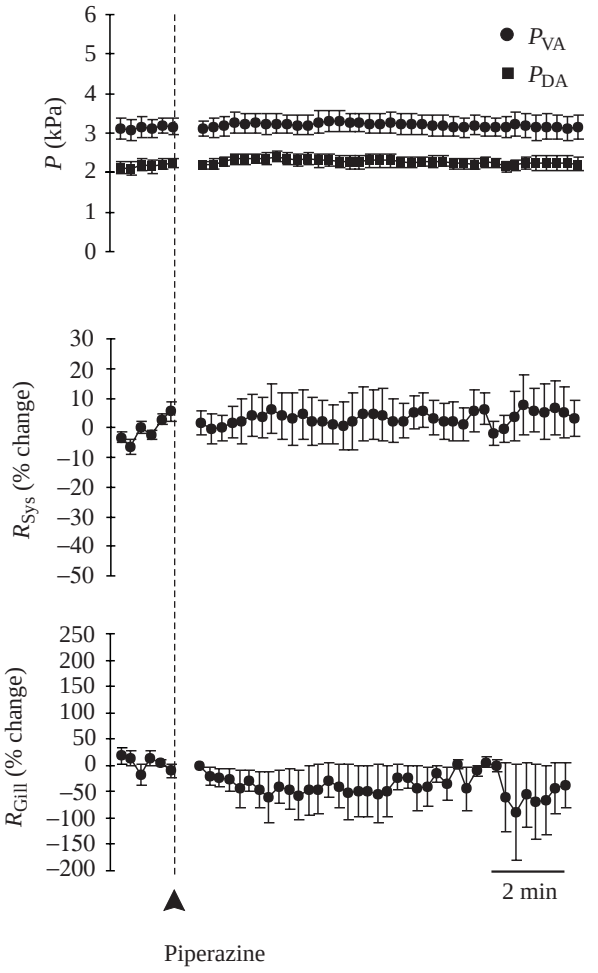

Fig. 4. The effects of intra-arterial injections of piperazine (a 5-HT_1 receptor agonist, 0.1 nmol kg^{-1}, N=7) on cardiovascular variables. Abbreviations as in Fig. 1. Values are mean ± S.E.M. There were no significant differences in the variables before and after injection of piperazine (upward-pointing arrowhead). The discontinuous traces in A are due to injection artefacts in the dorsal aortic pressure trace affecting the resistance calculations.

serotonin required to elicit similar significant cardiovascular responses between this species (0.1 nmol kg^{-1}) and rainbow trout (100 nmol kg^{-1}). The high potency of serotonin in borch gill vessels has also been demonstrated *in vitro* (Forster *et al.* 1998). The approximately 1000-fold higher sensitivity to serotonin in borch compared with rainbow trout may be due to species differences, but it could also represent adaptation to a cold environment. Cold adaptation has been suggested as an explanation for the dominance of α-adrenoceptor over β-adrenoceptor responses in borch (Axelsson *et al.* 1994) and in winter fish from temperate waters (Pärt *et al.* 1982; Randall and Perry, 1992). Interestingly, cooling enhances, presumably by an increasing in receptor affinity, both 5-HT_2-receptor- and α_2-adrenoceptor-mediated vasoconstriction in mammals (Vanhoutte and Flavahan, 1986; Bodelsson *et al.* 1990*a*,*b*; Harker *et al.* 1991). If a direct effect of temperature on receptor affinity applies to 5-HT_2 receptors in fish and if there is an equivalent receptor density among species, then fish should display different sensitivities to serotonin depending on the ambient water temperature.

A consequence of the serotonin-induced branchial vasoconstriction was an impaired gas transfer and, as for the vascular responses, the reduction in arterial P_{aO_2} could be blocked by methysergide treatment (Fig. 1B). Interestingly, the degree of reduction in P_{aO_2} depended on the resting P_{aO_2} (Fig. 2), i.e. animals with high resting levels displayed a larger reduction in P_{aO_2} following serotonin administration. A close correlation between an increased pressure differential (P_{VA}–P_{DA}) and P_{aO_2} has been noted in a study of the borch cardiovascular system (Forster *et al.* 1998). Assuming that the pressure difference across the gills represents gill vascular

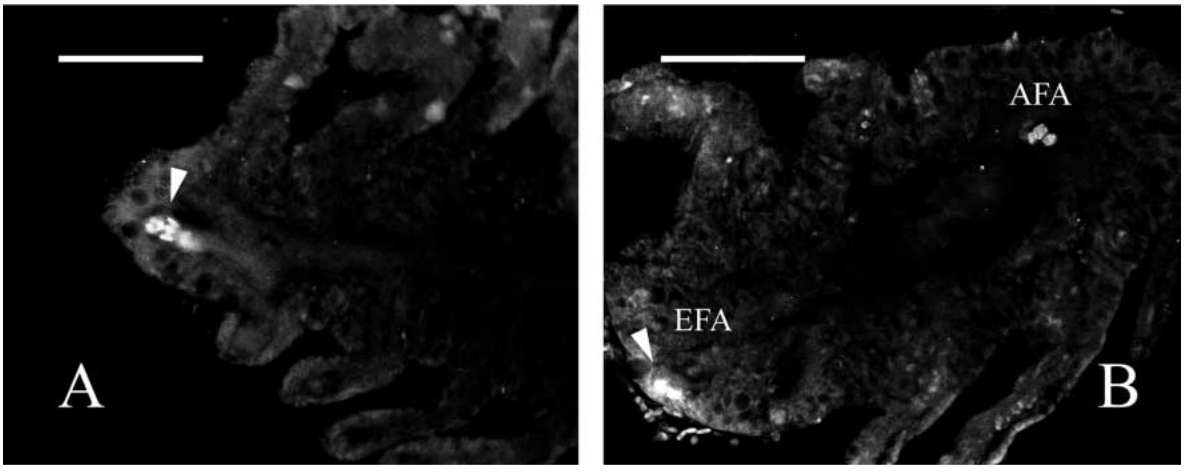

Fig. 5. Longitudinal (A) and transverse (B) sections through a gill filament of *Pagothenia borchgrevinki* showing 5-HT-immunoreactive (5-HT-IR) cells (indicated by arrowheads) on the efferent side of the filament. A group of 5-HT-IR cells forms a cluster near the filament tip (A); these cells are located on the outside of the efferent filamental artery (EFA) close to the external medium. AFA, afferent filamental artery. Scale bars, 100 μm.

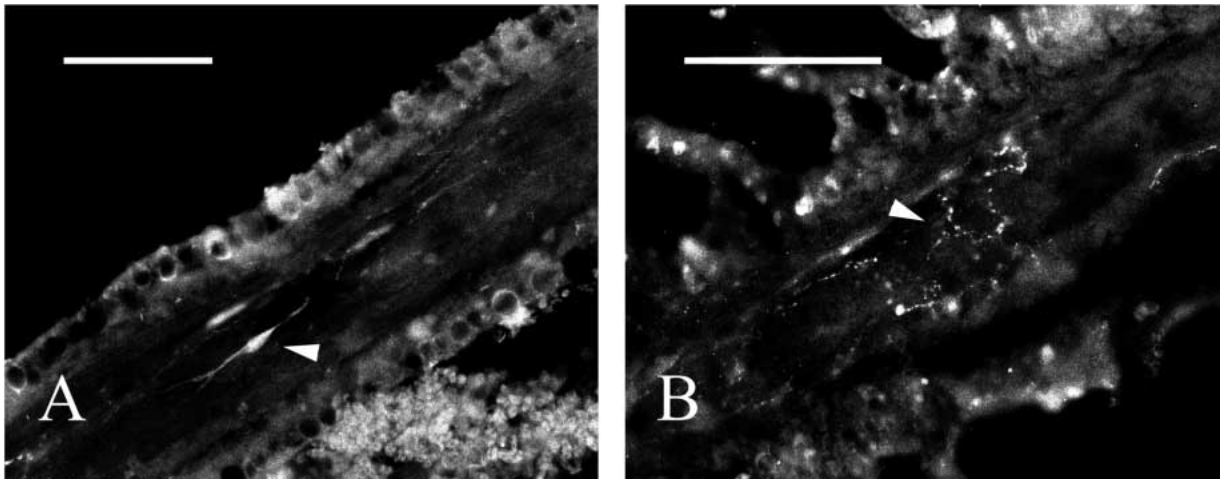

Fig. 6. Longitudinal sections through the middle of the efferent part of a *Pagothenia borchgrevinki* gill filament showing 5-HT-immunoreactive (5-HT-IR) bipolar neurones (A, arrowhead) and varicose 5-HT-IR nerve fibres (B, arrowhead). Scale bars, 100 μm.

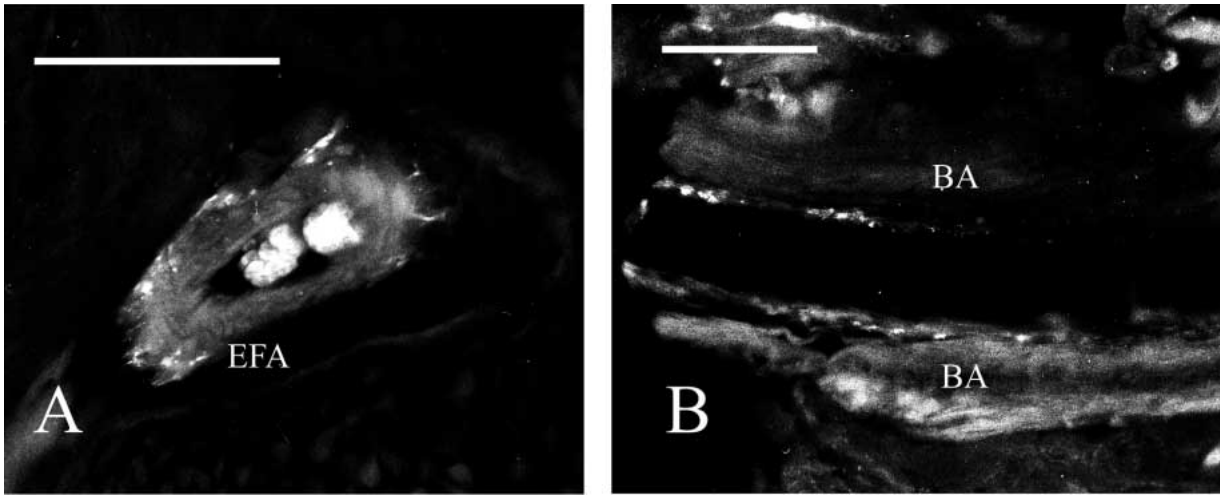

Fig. 7. Longitudinal sections through the basal parts of the gill filament (A) and the branchial arteries (B) of *Pagothenia borchgrevinki*. The section in A passes cut transversely through the efferent filamental artery (EFA). Note the rich innervation by 5-HT-immunoreactive (5-HT-IR) nerve fibres surrounding the EFA in A. The outer walls of the two branchial arteries (BA) in B are innervated by 5-HT-IR nerve fibres. Scale bars, 100 μm.

resistance, there appears to be a relationship between branchial resistance and dorsal aortic oxygen tension. In the present study, the correlation between the magnitude of the serotonin-induced decrease in $P_{a_{O_2}}$ and the resting $P_{a_{O_2}}$ may again indicate the importance of branchial vascular resistance for the oxygenation of arterial blood. Exogenous injection of serotonin similarly diminishes gas transfer in rainbow trout (Thomas *et al.* 1979; Fritsche *et al.* 1992).

The physiological significance of the effects of serotonin on gas transfer in teleosts is unclear, since serotonin is released from neuroepithelial cells in rainbow trout gills during hypoxic exposure (Dunel-Erb *et al.* 1982), and Bailly *et al.* (1989) suggested that serotonin-mediated constriction of the efferent arterial vasculature would cause an increase in perfusion pressure which would improve perfusion of the more distal lamellae, a mechanism thought to be essential during hypoxia. Therefore, the present results (Fig. 1B), like those in rainbow trout (Thomas *et al.* 1979; Fritsche *et al.* 1992), seem maladaptive. This conflict initiated a study on rainbow trout (Sundin *et al.* 1995) in which simultaneous observations of the microcirculation within the filaments and measurements of cardiovascular parameters were made. Serotonin induced a vasoconstriction in the filaments that redistributed blood flow towards the base of the filaments. Olson (1979) has suggested that the most efficient way to increase oxygen transfer across the gill is to recruit additional lamellae; however, the occlusion of the distal lamellae induced by serotonin would have the opposite effect. It has been shown that a serotonergic mechanism is not involved in hypoxia-induced branchial vasoconstriction in rainbow trout (Sundin and Nilsson, 1997).

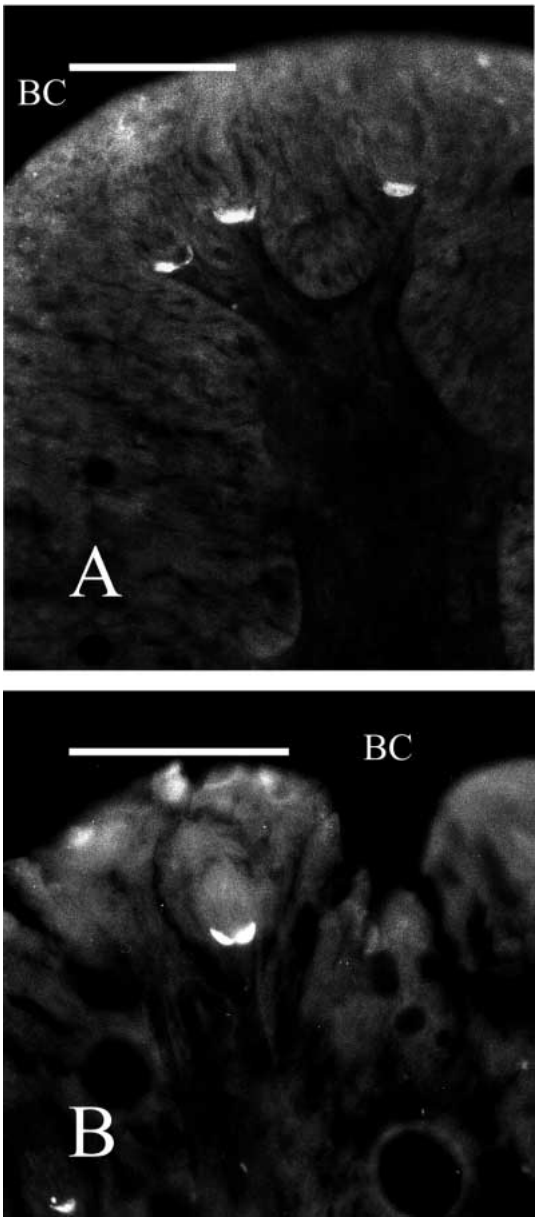

Fig. 8. Longitudinal section through the gill arch of *Pagothenia borchgrevinki* showing 5-HT-immunoreactive (5-HT-IR) cells in the gill rakers directed towards the buccal cavity (BC). Note that the stained cells are situated at the ends of the branches of a tree-like structure (A) and that the grouped cells (at least two, sometimes more) are connected like flower-cups (B). Scale bars, 100 μm.

Moreover, the water south of the Antarctic convergence is cold and well-oxygenated throughout the year; therefore, it is unlikely that Antarctic fish would be exposed to hypoxia. This, together with the fact that borch seem to be more sensitive to serotonin than are the temperate species tested, further argues against a hypoxic serotonergic vasoconstriction of the arterio-arterial pathway in fish. Other possible roles for serotonin in fish gills are in ventilatory (Fritsche *et al.* 1992; Janvier *et al.* 1996*a*), sensory (Dunel-Erb *et al.* 1982; Burleson and Milsom, 1995; Sundin, 1996) and acid–base (Thomas *et al.* 1979; L. Sundin and S. Nilsson, unpublished data) systems. It should also be remembered that 5-HT is normally released locally and, when injected into the circulation, it can bind to all available 5-HT receptors in the circulatory system causing more generalised effects than when it is released locally.

As in other teleosts (Dunel-Erb *et al.* 1982, 1989; Bailly *et al.* 1989, 1992; Zaccone *et al.* 1992; Sundin, 1996), and corroborating the physiological experiments discussed above, 5-HT-IR cells and nerve fibres were found within the gill tissues (Figs 5–8). As has been described in all other teleosts studied so far, the 5-HT-IR cells and nerve fibres were located on the efferent side of the filaments. 5-HT-IR nerves lining both arteries were detected (Fig. 7B). Therefore, unlike previous reports of an ‘exclusive’ location of serotonin storage sites on the efferent side of the filaments in fish gills (Dunel-Erb *et al.* 1982, 1989; Bailly *et al.* 1989, 1992; Zaccone *et al.* 1992; Sundin, 1996), the present study also provides evidence for a serotonergic innervation of the afferent branchial artery. Such innervation supports recent observations of serotonin-induced constriction of afferent branchial artery ring preparations in borch (Forster *et al.* 1998). Furthermore, despite the supposed exclusive location of efferent filamental serotonin storage sites, serotonin-induced constriction of an afferent filamental artery in rainbow trout has also been observed (Sundin *et al.* 1995). Evidently, there are receptors for serotonin on both sides of the branchial vasculature. If the finding of afferent branchial 5-HT-IR fibres in borch applies to other teleost species, the source providing serotonin to act on afferent receptors does not necessarily have to be extrabranchial, as has been discussed previously (Sundin *et al.* 1995). Although a direct vasoregulatory function of serotonin released from the different 5-HT-IR cells can be assumed, the localisation of the grouped neuroepithelial cells at the filamental tips in borch (Fig. 5A) implies a function unrelated to branchial vasomotor control. In accordance with the concept of water-sensing branchial oxygen receptors in rainbow trout (Bailly *et al.* 1992) and Atlantic cod *Gadus morhua* (Sundin, 1996), these grouped neuroepithelial cells may represent a site for environmental oxygen sensors. In addition, the peculiar 5-HT-IR cells on the gill rakers near the buccal cavity (Fig. 8) may also be part of a chemoreceptive mechanism.

As opposed to the branchial events, intra-arterial injections of serotonin induced a methysergide-insensitive decrease in R_{Sys} (Fig. 1), a response identical to that obtained in other teleosts (Sundin *et al.* 1995; Janvier *et al.* 1996*b*). This insensitivity to methysergide and the fact that specific 5-HT_1 and 5-HT_2 receptor agonists did not reduce R_{Sys} to a similar extent (Figs 3, 4) show that the mechanism responsible is not mediated by 5-HT_1/5-HT_2 receptors. Furthermore, the serotonin-induced systemic vasodilation in the European eel *Anguilla anguilla* is also insensitive to 5-HT_3 and 5-HT_4 receptor antagonists and cardiac vagotomy (Janvier *et al.* 1996*b*). In contrast to the lack of blockade of the inhibitory effect of serotonin on R_{Sys} in fish, methysergide is a potent antagonist of both endothelium-dependent and endothelium-

independent inhibitory serotonergic mechanisms in the rabbit and rat jugular vein (Leff *et al.* 1987; Martin *et al.* 1987; Bodelsson *et al.* 1993). This controversy adds complexity to the mechanisms behind the vasodilatory action of serotonin in fish.

In conclusion, the cardiovascular system of borch is extremely sensitive to serotonin and the branchial vasoconstriction is mediated by 5-HT_2 receptors. These facts, in conjuction with the immunohistochemical findings of both 5-HT-IR cells and 5-HT-IR nerve fibres within the gill tissues, demonstrate the presence of a serotonergic control system in the branchial vasculature of the Antarctic fish *P. borchgrevinki*. However, we cannot, at present, identify the mechanism responsible for the systemic serotonin-induced vasodilation.

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