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EFFECTS OF SEROTONIN ON CIRCULATION AND RESPIRATION IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Summary

The effects of serotonin (5-hydroxytryptamine; 5-HT) on continuously recorded dorsal and ventral aortic blood pressures, (P_{DA}, P_{VA}), arterial oxygen tension (Pa_{O_2}), arterial carbon dioxide tension (Pa_{CO_2}), extracellular pH (pHa), buccal pressure (P_{buccal}) and plasma catecholamine levels were investigated in rainbow trout, *Oncorhynchus mykiss*. Intra-arterial injections of serotonin (50–250 nmol kg^{-1}) caused a rapid decrease in P_{DA} and an increase in P_{VA}, suggesting vasoconstriction of the branchial vasculature. The blood pressure changes were accompanied by a reduction in Pa_{O_2} (approximately 3–8 kPa depending on the dose injected), an increase in Pa_{CO_2} (approximately 0.03–0.07 kPa) and a decrease in pHa (approximately 0.02–0.12 pH units). These changes, indicative of impaired gas transfer, occurred despite obvious hyperventilation based on measurements of buccal pressure. After pre-treatment with the serotonergic receptor antagonist methysergide, injections of 100 nmol kg^{-1} serotonin caused an increase in Pa_{O_2}, a reduction in Pa_{CO_2} and an increase in pHa. Methysergide treatment did not affect the usual serotonin-induced hyperventilation or the reduction in P_{DA} but did abolish the rise in P_{VA}; indeed, P_{VA} was lowered significantly by serotonin after methysergide treatment. This reduction in P_{VA} was eliminated by pre-treatment of fish with the combination of methysergide and sotalol (β-adrenoceptor antagonist), suggesting an adrenergic component to the overall blood pressure response. Analysis of plasma catecholamines after injection of serotonin revealed that high doses (50, 100, 250 nmol kg^{-1}) caused significant increases in concentrations of both noradrenaline and adrenaline.

We conclude that the cardio-respiratory effects of exogenous serotonin injections are complex and arise from several integrated responses, including the direct action of serotonin on receptors within the branchial and systemic vasculatures, indirect action on ganglionic receptors, and the stimulation of catecholamine release from adrenergic nerves and/or chromaffin cells.

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Key words: *Oncorhynchus mykiss*, serotonin, gills, circulation, respiration, catecholamines, gas transfer, ventilation.

Introduction

Histochemical studies have revealed the presence of serotonin (5-hydroxytryptamine; 5-HT) in the fish gill (e.g. Dunel-Erb *et al.* 1982, 1989; Bailly *et al.* 1989). At least two specific sites of storage have been identified: (i) neuroepithelial cells (NECs) within the filament epithelium (Dunel-Erb *et al.* 1982) and (ii) nerve fibres associated with the sphincter of the efferent filamental artery (Bailly *et al.* 1989). The NECs, which are usually isolated or found in clusters, are located near the filament epithelium basal lamina, mainly on the efferent side of the gills. The serotonin contained within these cells appears to be localized in dense-cored vesicles (Dunel *et al.* 1982). In rainbow trout (*Oncorhynchus mykiss*) acute, severe hypoxia was shown to elicit degranulation of the dense-cored vesicles (Dunel-Erb *et al.* 1982) which is indicative of serotonin release. This finding, when considered in conjunction with the known potent vasoconstrictory effects of serotonin on the gill vasculature (Östlund and Fänge, 1962; Katchen *et al.* 1976; Kent and Peirce, 1978), suggests a potential physiological role for serotonin during environmental hypoxia or at other times when serotonin is released in the gill. Indeed, in dogfish (*Squalus acanthias*) it was demonstrated that injections of serotonin mimic several of the physiological responses to hypoxia including an increase in branchial vascular resistance and bradycardia (Kent and Peirce, 1978). Despite the obvious potential effects of serotonin-induced branchial haemodynamic adjustments on gas transfer, we are unaware of any studies that have evaluated the effects of serotonin on gill respiratory gas exchange or blood respiratory status. Furthermore, the possible effects of serotonin on gill ventilation have yet to be assessed.

Thus, the present study was undertaken (i) to describe the *in vivo* effects of intra-arterial injections of serotonin simultaneously on circulation (pre- and postbranchial blood pressure, P_{VA}, P_{DA}) and respiration (buccal pressure, P_{buccal}, arterial oxygen tension, Pa_{O_2}, carbon dioxide tension, Pa_{CO_2}, and pHa) and (ii) to establish the underlying mechanisms.

Materials and methods

Experimental animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of either sex, weighing between 750 and 1200 g, were obtained from Linwood Acres Trout Farms (Campbellcroft, Ontario). Fish were held indoors in large fibreglass tanks supplied with flowing, aerated dechlorinated city of Ottawa tapwater ($[Na^+]$=0.12 mmol l^{-1}; $[Cl^-]$=0.15 mmol l^{-1}; $[Ca^{2+}]$=0.35–0.40 mmol l^{-1}; $[K^+]$=0.03 mmol l^{-1}; pH 7.5–8.0). The temperature of the holding and experimental tanks varied between 12 and 14 °C (April–June); photoperiod was kept constant at 12 h light: 12 h dark. Fish were fed daily but were not fed for 48 h prior to experimentation.

Surgical procedures

Series 1: extracorporeal circulation

The fish were anaesthetized in tapwater containing MS 222 (ethyl *m*-aminobenzoate,

1:10 000 w/v; adjusted to pH 7.5–8.0 with $NaHCO_3$) gassed with oxygen. The gills were irrigated with the same solution throughout the surgery. A polyethylene cannula (PE 50) was inserted into the dorsal aorta (Soivo *et al.* 1975) for measurements of dorsal aortic blood pressure (P_{DA}). A buccal cavity catheter was implanted using PE 160 tubing to measure changes in buccal pressure (P_{buccal}) associated with breathing movements. The fish was then placed on its left side and a small incision (2–3 cm) was made just behind the pectoral fin. The coeliac artery was dissected free and one cannula (PE 50) was inserted towards the heart for continuous sampling of blood. A similar cannula was inserted in the opposite direction to achieve an extracorporeal circuit in which the blood could flow continuously by means of a peristaltic pump (Thomas and Le Ruz, 1982). The cannulae were filled with 140 mmol l^{-1} NaCl and heat-sealed. After surgery, the fish were placed into individual opaque Perspex boxes (31 volume), supplied with Ottawa tapwater, and allowed to recover for 24 h prior to experimentation.

Series 2: blood samples for analysis of catecholamines

These fish were equipped with a dorsal aortic cannula only. The fish were left to recover in opaque Perspex boxes for 48 h before experimentation.

Series 3: ventral and dorsal aortic blood pressure measurements

Fish were anaesthetized in the same conditions as above and a dorsal aortic cannula was implanted accordingly. The fish was then placed on its right side and the third gill arch was exposed. The afferent branchial artery was cannulated by using a guitar string guide inside a PE 50 cannula. The cannulae were sutured to the skin. The fish were allowed to recover for 48 h before any blood pressure measurements were made. Strictly speaking, this protocol allows the measurement of afferent branchial artery blood pressure rather than ventral aortic blood pressure. However, owing to the lack of appreciable resistance to flow in the ventral aorta, this procedure does permit an accurate assessment of ventral aortic pressure and thus throughout this paper we refer to afferent branchial artery blood pressure as ventral aortic blood pressure.

Experimental protocol

Series 1: extracorporeal circulation

To prevent clotting, the tubing and chambers of the extracorporeal circuit were rinsed with heparinized physiological saline solution (1000 i.u. ml^{-1}). The cannulae in the coeliac artery were then connected in series with chambers holding the oxygen, carbon dioxide and pH electrodes. Blood flow in the circuit was established by a peristaltic pump (0.4 ml min^{-1}); the volume of blood contained in the extracorporeal circuit (0.5 ml) represented less than 2% of the total blood volume of the fish. The circuit allowed continuous recordings of arterial oxygen tension (Pa_{O_2}), carbon dioxide tension (Pa_{CO_2}) and pH (pHa) throughout the experiment.

The dorsal aortic and buccal cavity cannulae were connected to pressure transducers (Bell and Howell) for recording of blood pressure and buccal pressure on a physiograph chart recorder (Lafayette Instrument Company). The pressure transducers were calibrated

against a static column of water. In addition to the physiograph recordings, all variables (P_{DA}, P_{buccal}, Pa_{O_2}, Pa_{CO_2}, pHa) were sampled by a computer during the entire experiment. The software used was AD/DATA (P. Thoren, Department of Physiology, University of Göteborg, Sweden). Four samples per second were taken and means were calculated every second.

After stabilization of the recorded cardiovascular, ventilatory and blood respiratory variables (usually within 1 h after initiating the extracorporeal circuit), the experiment was commenced by injecting an initial dose of serotonin. In the first group of fish, dose–response relationships were established by injecting different doses of serotonin (1, 10, 50, 100 and 250 nmol kg^{-1} body mass). It was found that 100 nmol kg^{-1} was a suitable dose which gave clear, yet not maximal, effects on the recorded variables. Therefore, this dose was chosen for series 3. When the fish had recovered from the first dose of serotonin (as indicated by a return of all measured variables to the pre-injection values), the serotonergic receptor antagonist methysergide (1.5 mg kg^{-1}) was injected *via* the coeliac artery. Preliminary experiments established that effective serotonergic blockade was achieved after 30 min. At this time, another injection of serotonin was made (100 nmol kg^{-1}). In a control group of fish, saline (0.9 % NaCl) was injected instead of methysergide.

Series 2: blood samples for analysis of catecholamines

To test the hypothesis that serotonin stimulates release of catecholamines, either from adrenergic nerves or from chromaffin cells, blood (0.4 ml) was sampled before and 2, 5 and 15 min after injection of serotonin. Separate groups of fish were treated with 1, 10, 50, 100 or 250 nmol kg^{-1} of serotonin. An additional group was injected with saline (0.9 %) and served as a control for repeated blood sampling.

Series 3: ventral and dorsal aortic pressure measurements

The cannulae in the dorsal aorta and the afferent branchial arteries were connected to pressure transducers for recording of dorsal and ventral aortic blood pressure on a multi-channel physiograph (Harvard). Serotonin (100 nmol kg^{-1}) was injected *via* the dorsal aortic cannula. A pharmacological approach was used to assess the mechanisms underlying the effects of serotonin on the recorded cardiovascular variables. In separate groups of fish, different receptor antagonists were injected after the first serotonin injection and before a second injection of the same dose of serotonin. The second injection was made 1 h after the antagonist injection. The different groups were treated as follows: group 1: methysergide (4.2 μmol kg^{-1}: Sandoz); group 2: atropine (1.8 μmol kg^{-1}: Sigma), muscarinic cholinergic receptor antagonist; group 3: methysergide+atropine; group 4: sotalol (9.9 μmol kg^{-1}: Sandoz), β-adrenoceptor antagonist; group 5: methysergide+sotalol; group 6: phentolamine (5.3 μmol kg^{-1}: Ciba-Geigy), α-adrenoceptor antagonist.

Analytical procedures

Blood pH (pHa) was measured using a Metrohm EA 120 combination electrode in

conjunction with a radiometer PHM73 acid–base analyzer. Blood Pa_{O_2} and Pa_{CO_2} were measured by E5036-E5046 electrodes connected to a Radiometer PHM73 analyzer. The electrodes were calibrated by pumping saline equilibrated with appropriate gas mixtures obtained by Wösthoff pumps or buffer solution for the pH electrode. Measuring cells were kept at the same temperature as the fish by thermostatting the water.

Plasma adrenaline and noradrenaline levels were measured on alumina-extracted plasma samples using high performance liquid chromatography, HPLC (detector:Waters 460; column:Ultratech 50DS) in conjunction with electrochemical detection according to the basic method of Woodward (1982).

Statistical analysis

All data are presented as means ± 1 standard error of the mean (S.E.M.) Results have been statistically analysed using paired Wilcoxon signed-rank sum test ($P<0.05$). When variables were used in more than one paired comparison in the statistical evaluation, a sequentially rejective Bonferroni test (Holm, 1979) was used to minimize the chance of discarding any true null hypothesis.

Results

Series 1: extracorporeal circulation

Injections of 1 or 10 nmol kg^{-1} serotonin caused no significant changes in any of the recorded variables (data not shown). Injection of 50, 100 or 250 nmol kg^{-1} caused a rapid, dose-dependent decrease in P_{DA} (Fig. 1A). This was accompanied by hyperventilation as indicated by the large increase in P_{buccal} (Fig. 1B). Despite the hyperventilation, Pa_{O_2} decreased, Pa_{CO_2} increased and pHa fell (Fig. 2); the amplitude of these variations were dose-dependent. Clearly, gill respiratory gas transfer was impaired after injections of 50–250 nmol kg^{-1} serotonin. Pre-treatment with methysergide did not alter the effects of serotonin (100 nmol kg^{-1}) on P_{DA} or P_{buccal} (Fig. 1). However, the effects of serotonin on arterial blood respiratory status were reversed after pre-treatment with methysergide; Pa_{O_2} increased (Fig. 2A), Pa_{CO_2} decreased (Fig. 2B) and there was a trend (non-significant) towards increasing pHa (Fig. 2C). Fig. 3 is a representative continuous recording of Pa_{O_2}, Pa_{CO_2}, pHa, P_{DA} and P_{buccal} obtained using the extracorporeal circulation, which provides a more detailed description of the rapid and distinct phases of the response following an injection of serotonin (100 nmol kg^{-1}). The reduction in P_{DA} was immediate and reached its lowest value within 1 min. This initial decrease in P_{DA} was always followed by a rapid, and near total, reversal within 1–3 min of the injection (Fig. 3B). P_{DA} then decreased again to stabilize at intermediate levels during the 10–15 min interval before beginning a final phase of gradual recovery between 15 and 25 min after the injection. The transitory reversal in P_{DA} was always associated with an acceleration of the pHa reduction (Fig. 3), which was probably of metabolic origin because it was not accompanied by a corresponding increase in Pa_{CO_2}. This sequence of events was also consistently observed after injection of a 250 nmol kg^{-1} dose and was of larger amplitude (data not shown). After pre-treatment with methysergide (Fig. 4B) the transitory reversal in P_{DA} was prevented; progressive recovery started

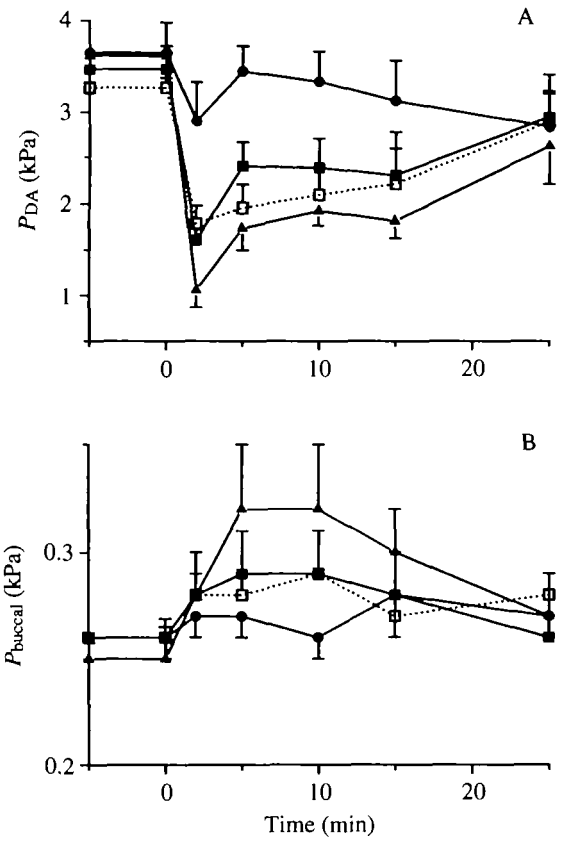

Fig. 1. The effects of serotonin (5-HT; 50 nmol kg^{-1}, ●; 100 nmol kg^{-1}, ■; 250 nmol kg^{-1}, ▲) injected into the dorsal aorta at time zero on (A) dorsal aortic blood pressure (P_{DA}) and (B) buccal pressure (P_{buccal}). The open symbols represent fish that were injected with 100 nmol kg^{-1} serotonin after pre-treatment with the serotonergic receptor antagonist methysergide. Values shown are means ±1 S.E.M. (N=6). Statistical analysis. the 0 min value was compared with the 2, 5 and 10 min value for each dose: P_{DA}: $P \geq 0.05$:(100) t=2,5; (250) t=2,5; P_{buccal}: $P \geq 0.05$:(100) t=2,5; (250) t=2,5. The 5 min value in the 100 group was compared with the 5 min value in the 100+methysergide group: no significant difference.

immediately and return to normal P_{DA} was usually achieved faster (compare Fig. 4A and 4B).

Series 2: blood sampling for analysis of catecholamines

Repeated blood sampling did not affect the levels of plasma catecholamines (Fig. 5, control) nor did injections of 1 or 10 nmol kg^{-1} of serotonin. However, injections of 50, 100 or 250 nmol kg^{-1} of serotonin provoked large increases in the plasma levels of both catecholamines (Fig. 5). Adrenaline and noradrenaline increased to similar levels after injection of serotonin, although the increase in plasma adrenaline concentration persisted for longer (Fig. 5).

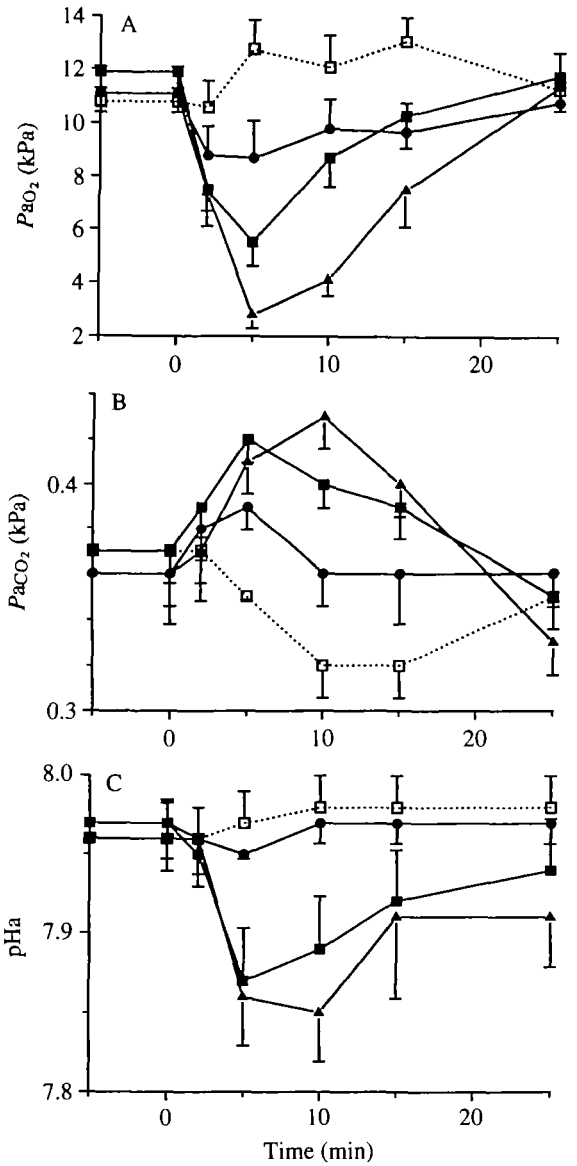

Fig. 2. The effects of serotonin (5-HT; 50 nmol kg^{-1}, ●; 100 nmol kg^{-1}, ■; 250 nmol kg^{-1}, ▲) injected into the dorsal aorta at time zero on (A) arterial blood oxygen partial pressure (P_{aO_2}), (B) arterial blood carbon dioxide partial pressure (P_{aCO_2}) and (C) arterial blood pH (pHa). The open symbols represent fish that were injected with 100 nmol kg^{-1} serotonin after pre-treatment with the serotonin receptor antagonist methysergide. Values shown are means ±1 S.E.M. (N=6). Same statistical comparisons as in Fig. 1. P_{aO_2}: (50) t=5; (100) t=2,5; (250) t=2,5; P_{aCO_2}: (100) t=5; (250) t=5,10; pHa: (100) t=5; (250) t=5. All three variables are different between the 100 and the 100+methysergide groups at 5 min.

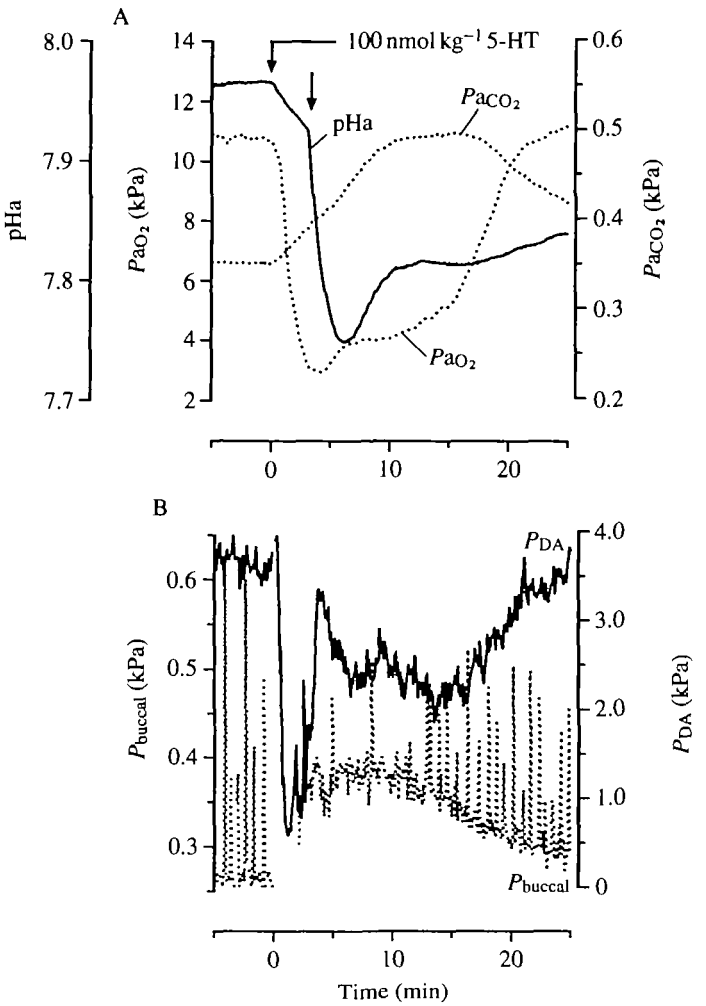

Fig. 3. A representative continuous recording of (A) arterial blood respiratory status (P_{aO_2}, lower dotted line; P_{aCO_2}, upper dotted line; pHa, solid line) and (B) cardio-respiratory variables (P_{DA}, solid line; P_{buccal}, dashed line) in rainbow trout, showing the effects of a single intra-arterial injection of serotonin (5-HT; 100 nmol kg^{-1}). Note the acceleration of the reduction in pHa (arrow in A) associated with the transitory reversal in P_{DA}. See text for further details. Note that the spikes in the P_{buccal} trace merely represent mechanical disturbances.

Series 3: ventral and dorsal aortic blood pressure measurements

As described above, injection of serotonin (100 nmol kg^{-1}) caused P_{DA} to decrease and pre-treatment with methysergide did not affect this response (Fig. 6, dashed line). P_{VA} was significantly increased by serotonin; pre-treatment with methysergide reversed the response to a significant decrease (Fig. 6).

Pre-treatment with the β-adrenoceptor antagonist sotalol (Fig. 7A) caused an increase in P_{VA}, even in the absence of serotonin. Injection of serotonin caused an additional

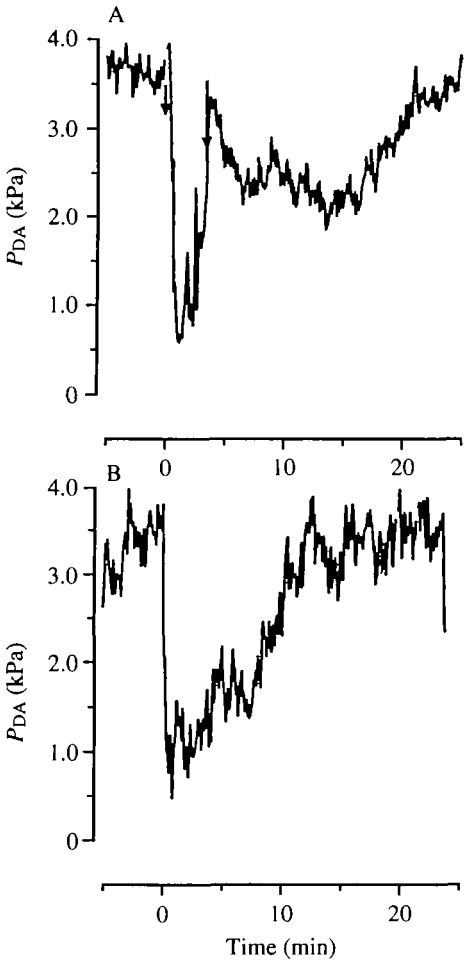

Fig. 4. Representative continuous recordings of dorsal aortic blood pressure (P_{DA}) in (A) control fish and (B) fish pre-treated with the serotonergic receptor antagonist methysergide showing the effects of a single intra-arterial injection of serotonin (5-HT; 100 nmol kg^{-1}). Note the absence of the transitory rebound in P_{DA} (delineated by the arrows in A) after pre-treatment of the fish with methysergide. See text for further details.

increase in P_{VA} which was significant at 2 min only; P_{DA} decreased to the same extent as in the non-treated group but remained lower for longer. When the fish were pre-treated with both sotalol and methysergide (Fig. 7B), the P_{DA} response to serotonin was unaffected but the P_{VA} response was different. Instead of decreasing, as after methysergide alone, P_{VA} was now unchanged. Pre-treatment with the muscarinic cholinergic receptor antagonist atropine or the α-adrenoceptor antagonist phentolamine did not significantly alter the effects of serotonin on P_{DA} or P_{VA} (data not shown).

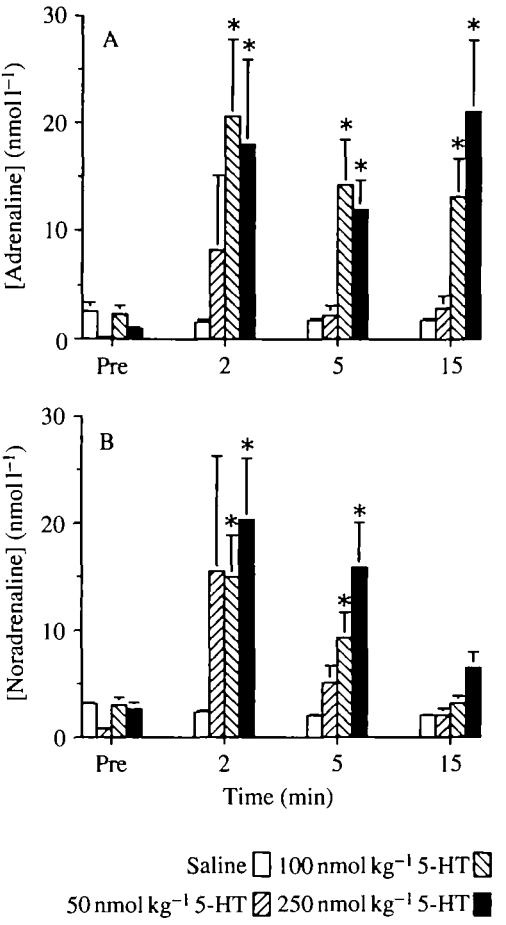

Fig. 5. The effects of saline or serotonin injections on the plasma levels of (A) adrenaline and (B) noradrenaline. * indicates a statistically significant difference from the pre-injection value (Pre). Values shown are means ±1 S.E.M.; N=6.

Discussion

Series 1 and 2

The present investigation clearly demonstrates that serotonin (of exogenous origin) has profound effects on branchial gas transfer at doses above 50 nmol kg^{-1}. The serotonin-induced decrease in P_{DA} and the concomitant increase in P_{VA} are indicative of branchial vasoconstriction and therefore suggest that the impairment of gas transfer was related to the vasoconstriction. The vasoconstriction presumably could occur in all areas within the gill where serotonergic receptors are located. A constrictory effect of serotonin on the branchial vasculature has previously been reported in perfused gill preparations (Östlund and Fänge, 1962; Reite, 1969; Katchen *et al.* 1976). Bailly *et al.* (1989) suggested that serotonin-mediated constriction of the efferent arterial vasculature would cause an increase in P_{VA} which would enhance perfusion of the more distal lamellae. Such a

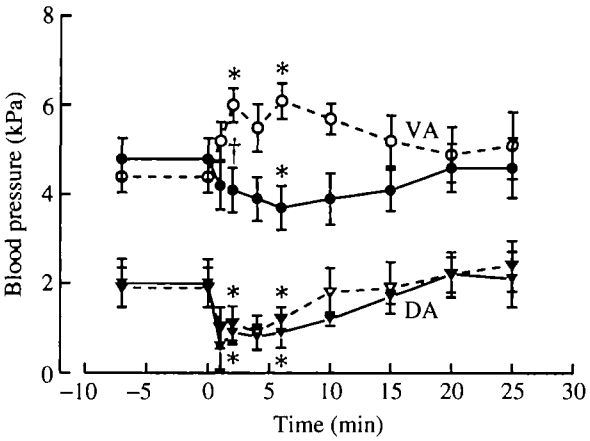

Fig. 6. The effects of intra-arterial injections of serotonin (100 nmol kg^{-1}) on ventral (VA) and dorsal (DA) aortic blood pressures, before (open symbols) and after (filled symbols) treatment with the serotonin receptor antagonist methysergide. * indicates a statistically significant difference between the pre-injection value (time=0) and the values 2 and 6 min after serotonin injection; † indicates a statistically significant difference between the value 2 min after serotonin injection before and after pre-treatment with methysergide. Values shown are means ±1 S.E.M., N=6.

mechanism would be of considerable importance during hypoxia. Thus, it has generally been assumed that the sites of serotonergic vasoconstriction are the efferent vessels of the arterio-arterial circulation. Recent evidence (Sundin and Nilsson, 1992) indicates that the precise site of vasoconstriction is the sphincter associated with the efferent filament artery. Based on the present data, however, the afferent side of the arterio-arterial circulation cannot be excluded as a site of serotonin-mediated vasoconstriction because such a response would be expected to reduce the functional lamellar surface area and consequently to impair gas transfer (e.g. Bergman *et al.* 1974). Vasoconstriction of the efferent blood vessels would affect P_{DA} and P_{VA} in a similar manner, but an impairment of gas transfer would be less likely. Indeed, constriction of the efferent gill vasculature is believed to enhance perfusion of distal lamellae and enhance gas transfer whereas the opposite response was observed in the present study.

Thus, although serotonin is released from neuroepithelial cells in the rainbow trout gills during hypoxic exposure (Dunel-Erb *et al.* 1982), the physiological significance of this response is unclear. It is conceivable that the release of endogenous serotonin during hypoxia could elicit fundamentally different responses from those observed after injections of exogenous serotonin during normoxia. Indeed, serotonin did evoke two other physiologically adaptive responses in addition to the apparent non-adaptive branchial vasoconstriction. These included hyperventilation and elevation of plasma catecholamine levels (see below). The hyperventilation was evidently unable to offset the deleterious effects of vasoconstriction on gas transfer. Thomas *et al.* (1979) reported that exposure of rainbow trout to acidic water caused a decrease in P_{aO_2} and pHa and increased ventilation and that these effects were reversed by pre-treatment with methysergide. These authors speculated that serotonin was released during acidosis and

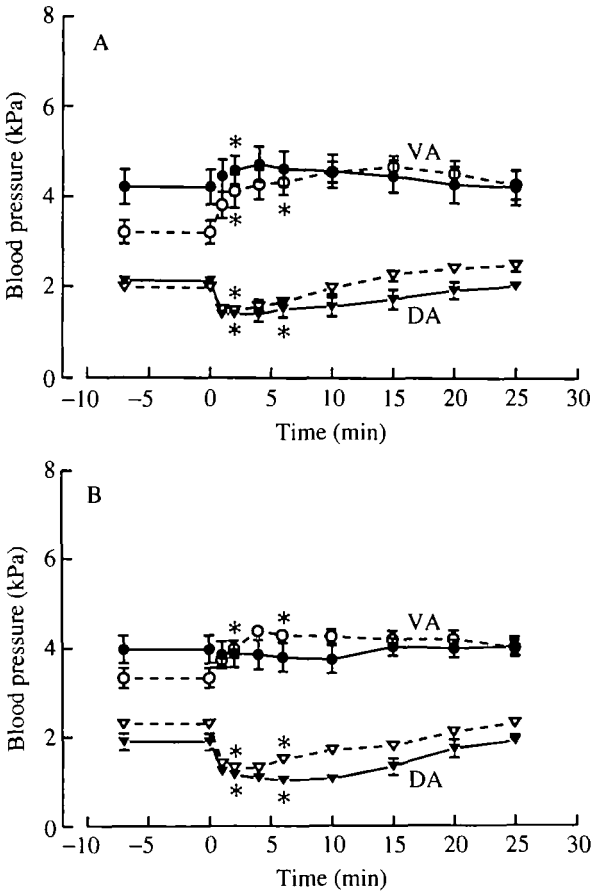

Fig. 7. (A) The effects of intra-arterial injections of serotonin (100 nmol kg^{-1}) on ventral (VA) and dorsal (DA) aortic blood pressures, before (open symbols) and after (filled symbols) treatment with the β-adrenoceptor antagonist sotalol. (B) The effects of intra-arterial injections of serotonin (100 nmol kg^{-1}) on ventral (VA) and dorsal (DA) aortic blood pressure, before (open symbols) and after (filled symbols) treatment with methysergide+sotalol. See Fig. 6 for statistical comparisons. Values shown are means ±1 S.E.M., N=6.

that its constrictory effect on the gill vasculature was responsible for the effects on blood respiratory status. The results of the present study support this contention. Further, by continuous recording of cardiovascular and blood respiratory variables, we have observed frequent, brief episodes of spontaneous hyperventilation, reduction in P_{DA} and impairment of gas transfer in untreated rainbow trout (S. Thomas, R. Fritsche and S. F. Perry; in preparation). This sequence of events was especially prevalent upon exposure of fish to gradual hypoxia. The remarkable similarity of these phenomena with the response pattern observed after injection of serotonin suggests a physiological role for this hormone which requires further elucidation.

The observation that methysergide reversed the effect of serotonin on blood respiratory status and on P_{VA}, but caused no change in the P_{DA} and ventilatory responses, indicates

that hyperventilation was not a consequence of the reduction in $P_{a_{O_2}}$. Furthermore, the increase in $P_{a_{O_2}}$ and the decrease in $P_{a_{CO_2}}$ suggest an enhancement of gas transfer consistent with the hyperventilation; thus, it is likely that methysergide blocked the branchial vasoconstriction and that the persistent reduction in P_{DA} was related to other mechanisms, such as serotonin-mediated (methysergide-insensitive) systemic vasodilation, which was unmasked by methysergide treatment. If the blood–brain barrier in trout is permeable to serotonin as it is in eels (Genot *et al.* 1981) then an effect of serotonin acting on receptors (unblocked by methysergide) within the central nervous system cannot be excluded. The elevation of plasma catecholamine levels after injection of serotonin and the potential interrelationships between serotonergic and adrenergic neurones adds complexity to the interpretation of the results. Serotonergic neurones have been shown to interact with adrenergic neurones to cause a release of transmitter substance acting on β-receptors (Fänge *et al.* 1976). Serotonergic neurones also display synaptic contact with catecholaminergic nerve fibres within the gills, suggesting a modulatory relationship between the sympathetic and cranial autonomic nerves supplying the teleost gill (Bailly *et al.* 1989). Elevation of plasma catecholamine levels normally elicits gill vasodilation (Nilsson and Pettersson, 1981; see also reviews by Nilsson, 1984, 1986) and a rapid increase in P_{DA}. Figs 3 and 4 show that after an injection of serotonin the decrease in P_{DA} was immediately followed by a transitory reversal. This relative and transient increase in P_{DA} may reflect the serotonin-mediated release of catecholamines. Moreover, in the presence of elevated levels of catecholamines, the permeability of the trout red blood cell (RBC) to Na^+ is suddenly increased by stimulation of a plasma membrane Na^+/H^+ antiporter, allowing the downhill entry of Na^+ in exchange for internal H^+ (see review by Thomas and Perry, 1992). Considering the high levels of both adrenaline and noradrenaline in the plasma (Fig. 5), it is likely that the RBC Na^+/H^+ antiporter was activated after injections of 100 and 250 nmol kg^{-1} serotonin. The acceleration of the pHa reduction (Fig. 3) at the time of the transitory increase in P_{DA} can thus be explained by adrenergic extrusion of H^+ from the RBC into the plasma. The fact that the rebound in P_{DA} was absent after pre-treatment with methysergide may indicate that the release of catecholamines was mediated by serotonergic, methysergide-sensitive receptors or that the decrease in $P_{a_{O_2}}$ occurring in untreated animals was a prerequisite for activation of the Na^+/H^+ exchanger as a result of the known effects of hypoxia on increasing the responsiveness of the RBC Na^+/H^+ antiporter to catecholamines (Motais *et al.* 1987).

Series 3

Acetylcholine is known to elicit branchial vasoconstriction (e.g. Bergman *et al.* 1974; Smith, 1977; Booth, 1979). It is unlikely, however, that the branchial vasoconstriction associated with the injection of serotonin was caused by interaction between serotonin and cholinergic nerve fibres because pre-treatment of fish with atropine did not significantly alter the blood pressure responses. The finding that P_{VA} decreased when serotonin was injected into fish pre-treated with methysergide, but remained unaffected after the combined pre-treatment with methysergide+sotalol, implies a direct effect of serotonin on the branchial vasculature (blocked by methysergide) as well as a secondary

adrenergic effect acting *via* β-receptors (unmasked by methysergide and blocked by sotalol) leading to vasodilation and hence the decrease in P_{VA}. Since serotonin caused the circulating levels of adrenaline and noradrenaline to increase, a direct effect of humoral catecholamines on branchial β-adrenoceptors could be responsible for the vasodilation. Alternatively, serotonin may have influenced branchial adrenergic neurones acting *via* β-adrenoceptors on the gill vasculature. Clearly, the vasodilatory effect is normally masked by the more prominent vasoconstriction. The mechanisms involved in the persistent decrease in P_{DA} after treatment with methysergide and the other receptor antagonists used in this study remains unresolved. It could either be due to the presence of methysergide-insensitive serotonergic receptors on the systemic vasculature or to the indirect effect of serotonin on a secondary neurone (non-cholinergic, non-adrenergic; Grove *et al.* 1974; Fänge *et al.* 1976). Also, the reduction in P_{DA} after methysergide treatment could be explained by an unmasking of systemic β-adrenoceptors (because methysergide is a weak α-adrenoceptor antagonist) coupled with an increase in plasma catecholamine levels. In mammals, many different subtypes of serotonergic receptors are found (Martin *et al.* 1987; Hartig and Lever, 1990).

The possibility that gill serotonin stored within neurones and/or neuroepithelial cells may be involved in sensory mechanisms and the triggering of nervous reflexes similar to those seen during hypoxia also complicate interpretation of the results. Thus, it is conceivable that some of the effects of serotonin injections *in vivo* may reflect the initiation of complex reflexes (Dunel-Erb *et al.* 1982, 1989; Bailly *et al.* 1989; Burleson, 1991; Fritsche and Nilsson, 1992). For example, the ventilatory responses to serotonin, which remained after pre-treatment with methysergide, could result from a reflex triggered by serotonin acting on sensory receptors.

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