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CONSEQUENCES OF CATECHOLAMINE RELEASE ON VENTILATION AND BLOOD OXYGEN TRANSPORT DURING HYPOXIA AND HYPERCAPNIA IN AN ELASMOBRANCH (*SQUALUS ACANTHIAS*) AND A TELEOST (*ONCORHYNCHUS MYKISS*)

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Summary

The marine dogfish (*Squalus acanthias*) and the seawater-adapted rainbow trout (*Oncorhynchus mykiss*) were exposed to acute environmental hypercapnia or hypoxia to evaluate (i) the dynamics of catecholamine release into the circulation and (ii) the impact of catecholamine release on gill ventilation and blood oxygen transport. This comparison was undertaken to test the hypothesis that the pattern and consequences of catecholamine release differ in the two species according to the presence or absence of a Root effect and a red blood cell (rbc) β-adrenergic response.

Hypercapnia and hypoxia elicited marked increases in plasma catecholamine levels in the trout but not in the dogfish. In the trout, catecholamine release occurred abruptly during hypoxia when arterial P_{O_2} (Pa_{O_2}) decreased below 2.7 kPa. In the dogfish, plasma catecholamine levels remained stable during hypoxia even when Pa_{O_2} fell below 2.0 kPa.

Trout and dogfish displayed pronounced hyperventilatory responses during both hypercapnia and hypoxia. In trout, the hyperventilatory response consisted of an increase in ventilation amplitude (estimated by opercular cavity pressure changes) with no change in ventilation frequency (f_V), whereas in the dogfish, both amplitude (estimated by spiracular cavity pressure changes) and f_V increased significantly. The use of an extracorporeal circulation and frequent blood sampling demonstrated that plasma catecholamine levels and ventilation amplitude were not correlated during hypoxia in either species.

During hypercapnia in trout, the bolus injection of a catecholamine cocktail (final nominal circulating levels 200 nmol l^{-1} adrenaline, 50 nmol l^{-1} noradrenaline) caused a rapid (within 2 min) 33 % reduction in ventilation amplitude that persisted for 3 min; f_V was unaffected. This hypoventilatory response occurred concurrently with activation of rbc Na^+/H^+ exchange and an increase in arterial blood O_2 content (Ca_{O_2}) and O_2 specifically bound to haemoglobin (O_2/Hb). During hypoxia in trout, a similar injection of catecholamines activated rbc Na^+/H^+ exchange and increased O_2/Hb yet was without effect on ventilation amplitude or f_V. In dogfish during hypercapnia or hypoxia, injection of a catecholamine cocktail (final nominal circulating levels 125 nmol l^{-1} adrenaline, 125 nmol l^{-1} noradrenaline) caused slight but significant reductions in f_V (3–4 min^{-1}) without affecting ventilation amplitude. Catecholamine injections did not affect blood oxygen transport in dogfish.

The results demonstrate significant differences in the nature of catecholamine release in dogfish and trout that may reflect, in part, the absence of a Root effect and rbc adrenergic Na^+/H^+ exchange in the elasmobranch. The present data do not support the hypothesis that circulating catecholamines play a major role in controlling breathing during hypoxia or hypercapnia.

Key words: *Oncorhynchus mykiss*, *Squalus acanthias*, catecholamines, ventilation, hypoxia, hypercapnia, blood oxygen transport.

Introduction

In response to acute stress, numerous fish species release the catecholamine hormones adrenaline and noradrenaline into their circulation (reviewed by Randall and Perry, 1992; Thomas and Perry, 1992; Gamperl *et al*. 1994). This adrenergic response is thought to evoke an array of compensatory physiological and metabolic adjustments aimed at relieving the

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deleterious consequences associated with the stress episode (reviewed by Nilsson, 1984, 1994; Perry and Wood, 1989; Randall and Perry, 1992; Thomas and Perry, 1992). The origin of the circulating catecholamines varies amongst fish species. In teleosts, the major source is the chromaffin tissue associated with the posterior cardinal vein/head kidney (Nandi, 1961; Nilsson, 1983; Santer, 1994) whereas, in elasmobranchs, catecholamines within the circulation are derived predominantly from the axillary bodies of the sympathetic ganglia (Nilsson, 1983; Santer, 1994).

The patterns and mechanisms of catecholamine release during acute stress have been studied extensively in the teleosts in comparison with the elasmobranchs. In both groups, however, the predominant trigger for catecholamine secretion is believed to be the release of the neurotransmitter acetylcholine from preganglionic cholinergic fibres of the sympathetic nervous system (Nilsson *et al.* 1976; Abrahamsson, 1979; Opdyke *et al.* 1983; reviewed by Randall and Perry, 1992).

On the basis of several recent studies, a model has emerged for the control of catecholamine release in teleosts in which depression of blood oxygen content (or a closely related variable such as haemoglobin oxygen-saturation) is the proximate stimulus eliciting secretion during acute hypercapnia (Perry *et al.* 1989) or hypoxia (Thomas and Perry, 1992; Thomas *et al.* 1992; Randall and Perry, 1992; Perry and Reid, 1992, 1994). For example, during hypercapnia in rainbow trout (*Oncorhynchus mykiss*), the release of catecholamines is not induced specifically by respiratory acidosis but instead is related to the hypoxaemia caused by the Root effect (Perry *et al.* 1989). Furthermore, the elevation of circulating catecholamine levels caused by intra-arterial injection of acid (Boutilier *et al.* 1986) is abolished under conditions of hyperoxia (Aota *et al.* 1990). Indeed, there is no direct evidence to implicate acidosis, *in itself*, as a stimulus for catecholamine release in teleosts exhibiting Root effects. During hypoxia in trout, plasma catecholamine levels remain stable until a critical P_{O_2} threshold is reached corresponding to 50–60 % Hb O_2-saturation (Thomas and Perry, 1992; Thomas *et al.* 1992; Perry and Reid, 1992, 1994). Thus, during both hypercapnia and hypoxia in trout, catecholamine release is closely linked to blood oxygenation status. The significance of such a linkage in trout and other active teleosts is obvious, given the important role of catecholamines in regulating blood oxygen transport, and arises largely, though not exclusively, from β-adrenergic activation of red blood cell (rbc) Na^+/H^+ exchange (reviewed by Nikinmaa, 1992; Nikinmaa and Tufts, 1989).

Elasmobranchs lack both a Root effect (see review by Butler and Metcalfe, 1988) and an rbc β-adrenergic Na^+/H^+ exchanger (Tufts and Randall, 1989; Wood *et al.* 1994), but are believed to rely exclusively on circulating catecholamines for adrenergic cardiovascular control (reviewed by Butler and Metcalfe, 1988) because of the absence of innervation of the heart or systemic vasculature by sympathetic nerves (Nilsson, 1983). Thus, the pattern of catecholamine release in elasmobranchs during hypercapnia and hypoxia might differ substantially from that in the active teleosts. In addition, because elasmobranchs lack an rbc β-adrenergic Na^+/H^+ exchanger, the role of catecholamines on blood O_2 transport is unclear. Thus, the goals of the first component of this study were to compare the dynamics of catecholamine release and the involvement of circulating catecholamines in blood oxygen transport in dogfish (*Squalus acanthias*) and rainbow trout under conditions of acute hypercapnia or hypoxia.

The second component of this study addresses the consequences of catecholamine release on ventilation and blood oxygen transport in the dogfish and trout. Currently, the role of circulating catecholamines in stimulating ventilation during acute stress in fishes is debated because there is experimental evidence both for (Peyreaud-Waitzenegger, 1979; Aota *et al.* 1990; Aota and Randall, 1993; Burleson and Milsom, 1995; reviewed by Randall and Taylor, 1991) and against (Kinkead *et al.* 1991; Kinkead and Perry, 1990, 1991; reviewed by Perry *et al.* 1992) their involvement. It has been suggested (Randall and Taylor, 1991) that increases in ventilation during stress in dogfish are mediated *exclusively* by elevated levels of circulating catecholamines. Thus, a second goal of this study was to evaluate the involvement of circulating catecholamines in ventilatory control in dogfish and trout during hypercapnia and hypoxia.

Materials and methods

Experimental animals

Pacific spiny dogfish (*Squalus acanthias*) were collected by net during trawls by local fishermen and transported to holding facilities at Bamfield Marine Station (BMS; Bamfield, Vancouver Island, British Columbia). They were kept unfed under natural photoperiod in opaque circular tanks provided with aerated full-strength sea water (12 °C). In the present study, 20 dogfish (mass 1828±109 g, mean ± S.E.M.) were used within 4 weeks of their capture.

Freshwater rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were obtained from a local hatchery and transported to BMS where they were acclimated gradually (2 weeks) to full-strength sea water (12 °C) and kept unfed in outdoor holding tanks. In the present study, 11 trout (average mass 706±29 g) were used within 4 weeks following their acclimation.

Surgical procedures

Dogfish were anaesthetised in a seawater solution of ethyl-*m*-aminobenzoate (0.1 g l^{-1}; MS-222; Syndel) and transferred to an operating table where the gills were irrigated continuously with the same anaesthetic solution. After making a ventral incision and externalising much of the viscera, the coeliac artery was cannulated bi-directionally using polyethylene tubing (Clay Adams PE 50) filled with heparinised (100 i.u. ml^{-1} ammonium heparin) dogfish saline (500 mmol l^{-1} NaCl). The viscera were re-inserted, the wound sutured, and the cannulae were secured firmly to the ventral

musculature. In order to assess ventilatory amplitude and sample inspired water, catheters (Clay Adams PE 160) were inserted into the spiracular cavities and sutured to the head. After surgery, dogfish were placed into individual wooden or Perspex boxes provided with aerated full-strength sea water at ambient temperature (12 °C). Fish were left to recover for approximately 24 h prior to experimentation.

Rainbow trout were anaesthetised (see above) and transferred to an operating table for implantation of polyethylene cannulae (Clay Adams PE 50) in the dorsal aorta (Soivio *et al.* 1975) and caudal vein (see Axelsson and Fritsche, 1994). Cannulae were filled with heparinised teleost Cortland saline (Wolf, 1963) modified for seawater conditions (Perry *et al.* 1984). To assess ventilatory amplitude and sample inspired water, polyethylene catheters (Clay Adams PE 160) were implanted into the opercular cavities. After surgery, fish were permitted to recover for approximately 24 h in individual opaque Perspex boxes provided with aerated full-strength sea water (12 °C).

Experimental protocol

All experiments were performed using extracorporeal arterial blood shunts (Thomas and Le Ruz, 1982; Thomas, 1994) that permitted continuous and simultaneous measurements of arterial blood pH (pHa), oxygen partial pressure (P_{aO_2}) and carbon dioxide partial pressure (P_{aCO_2}). In the dogfish, this was achieved by pumping (peristaltic pump; 0.55 ml min^{-1}) blood from the coeliac artery (*via* the upstream cannula) through pH, P_{CO_2} and P_{O_2} electrodes connected in series and returning it to the fish *via* the downstream cannula in the coeliac artery. In the trout, arterial blood was pumped from the dorsal aortic cannula and returned *via* the caudal vein cannula. The extracorporeal shunt contained approximately 1.0 ml of blood, representing less than 1 % and 3 % of the total blood volume in dogfish and trout, respectively.

Immediately prior to experimentation, the extracorporeal shunt was rinsed for 20–30 min with a solution of ammonium heparin (540 i.u. ml^{-1}) to prevent blood from clotting in the tubing and electrode chambers. After starting the extracorporeal circulation, a period of approximately 10–15 min was required to achieve stable baseline recordings of pHa, P_{aCO_2} and P_{aO_2}. Upon stabilisation, experiments commenced with a period of normocapnic normoxia followed by either hypercapnia or hypoxia.

Normocapnic normoxia

Fish were monitored during an initial period of normocapnic normoxia during which time the inflowing water (>2.5 l min^{-1}) was passed through a gas equilibration column being gassed vigorously with air. After a suitable period of recording (approximately 15 min) of the blood gas (pHa, P_{aCO_2}, P_{aO_2}) and ventilatory (frequency, f_V; amplitude, V_{amp}) variables, an arterial blood sample (0.6 ml) was taken from the shunt for immediate analysis of total O_2 content (C_{aO_2}), haemoglobin concentration ([Hb]), and haematocrit (Hct). Plasma (>200 µl) was obtained by centrifugation (30 s, 12 000 ***g***), combined with 10 µl of a 5 % EDTA/10 % sodium bisulphite solution, quick-frozen in liquid N_2, and stored at −80 °C until subsequent analysis of catecholamine levels.

Hypercapnia

External hypercapnia was achieved by gassing the equilibration column with mixtures of CO_2 in air ranging between 3 and 10 %; these mixtures were provided by a Wösthoff gas-mixing pump (model M301 A/F). We did not have the capacity to measure water P_{CO_2} in these experiments, and thus the goal was to reach an arterial P_{CO_2} of 1.00–1.13 kPa within 10 min. This was accomplished by careful adjustments of water flow through the equilibrium column and the percentage CO_2 supplied by the Wösthoff pump.

After the blood gas variables had stabilised (usually within 20 min), f_V was assessed and a second sample was taken from the extracorporeal shunt and analysed as above.

Hypoxia

External hypoxia was achieved by gassing the equilibration column with N_2 to achieve water P_{O_2} (P_{wO_2}) levels of 4.7 kPa (dogfish) to 6.0 kPa (trout) within 30 min. On the basis of preliminary experiments, these P_{wO_2} values were chosen so as to elicit a lowering of C_{aO_2} in each species by approximately 50 %.

Upon initiation of hypoxia, blood samples (0.4 ml) were taken frequently from the extracorporeal loop at approximately 1.3 kPa intervals of P_{aO_2} for subsequent analysis of plasma catecholamine levels. The number of blood samples taken varied between four and nine per fish.

Upon reaching the target P_{wO_2}, f_V was assessed and a final blood sample was taken from the extracorporeal shunt and analysed for C_{aO_2}, [Hb], Hct and plasma catecholamine levels.

Catecholamine injections during hypercapnia and hypoxia

A catecholamine cocktail (0.5 ml kg^{-1}) was injected into the return cannula of the extracorporeal circulation during hypercapnia or hypoxia to mimic the levels of adrenaline and noradrenaline anticipated in the arterial circulation during acute severe stress (Butler *et al.* 1986; Randall and Perry, 1992; Gamperl *et al.* 1994). In dogfish, the nominal levels of adrenaline and noradrenaline immediately after injection were each estimated to be 125 nmol l^{-1}, whereas in the trout, the nominal levels of adrenaline and noradrenaline after injection were estimated to be 200 and 50 nmol l^{-1}, respectively. These estimates were calculated assuming that the catecholamines were being distributed rapidly within the extracellular fluid volume (300 ml kg^{-1}). For dogfish, the catecholamine cocktail was prepared using dogfish Ringer, and consisted of 7.5×10^{-5} mol l^{-1} L-adrenaline bitartrate (Sigma) and 7.5×10^{-5} mol l^{-1} L-noradrenaline bitartrate (Arterenol, Sigma). For trout, the catecholamine cocktail was prepared using modified Cortland saline and consisted of 1.2×10^{-4} mol l^{-1} adrenaline bitartrate and 3.0×10^{-5} mol l^{-1} noradrenaline bitartrate. The catecholamine stock solutions were kept frozen at −80 °C and were thawed immediately prior to being injected.

After the injection, blood respiratory variables and ventilation were assessed for 5 min, at which time a final blood sample was withdrawn from the extracorporeal loop and analysed for Ca_{O_2}, [Hb] and Hct.

Analytical procedures

Arterial blood pH, P_{CO_2} and P_{O_2} were monitored using Radiometer (CO_2, O_2) and Metrohm (pH) electrodes housed in thermostatted cuvettes and connected to a Radiometer PHM 73 meter. Water P_{O_2} was measured by continuous siphoning of inspired water from the opercular/spiracular catheters using another Radiometer O_2 electrode connected to a dual-channel O_2 meter (Cameron Instruments). The O_2 electrodes were calibrated by pumping (using the peristaltic pump of the extracorporeal shunt) a zero-oxygen solution (2 % sodium sulphite) or air-saturated water continuously through the electrode sample compartments until stable readings were recorded. The CO_2 electrode was calibrated in a similar manner using mixtures of 0.5 % and 1.0 % CO_2 in air provided by two Wösthoff gas-mixing pumps connected in series. The pH electrode was calibrated using Radiometer precision buffers. The CO_2, O_2 and pH electrodes were calibrated prior to each experiment.

Ventilation amplitude (V_{amp}) was assessed by monitoring spiracular (dogfish) or opercular (trout) pressure changes associated with the breathing cycle. The spiracular and buccal catheters were filled with sea water and connected to a pressure transducer (Bell and Howell) linked to an amplifier/physiograph (Harvard). The pressure transducer was calibrated daily against a static column of water. f_V was determined from the physiograph paper traces.

The extracorporeal circulation, ventilation catheters and arrangement of electrodes permitted the continuous and simultaneous measurements of pHa, P_{aCO_2}, P_{aO_2}, P_{wO_2} and V_{amp} during all experiments. The analog outputs from the meters and physiograph were transformed into digital data using a commercial A/D converter (Data Translation Incorporated). The digital data were then acquired by microcomputer using customised data-acquisition software (written by P. Thoren, Göteborg, Sweden). Mean values for each parameter were captured by computer and stored at 5 s intervals.

Ca_{O_2} was measured according to the method of Tucker (1967) on 40 µl samples using a Radiometer O_2 electrode and meter. [Hb] was determined spectrophotometrically on duplicate 20 µl samples using a commercial assay kit (Sigma). Haematocrit was determined on quadruplicate samples by centrifugation (10 min) at 5000 ***g***.

Plasma catecholamine levels were determined on alumina-extracted samples by HPLC (Varian) with electrochemical detection (E.G. & G. Parc, model 400 EC detector) according to the basic method of Woodward (1982). 3,4-Dihydroxybenzylamine was used as an internal reference standard in all analyses.

Statistical analyses

Data in tables and figures are presented as mean values ± 1 standard error of the mean (S.E.M.). The data were statistically analysed by parametric repeated-measures one-way analysis of variance (ANOVA). When the ANOVA indicated a significant difference, a *post-hoc* multiple-comparison test (Dunnett's test) was used to identify data points that were significantly different from a single control point (i.e. pre-hypercapnia, pre-hypoxia, pre-catecholamine injection). When parametric test assumptions were violated, the data were analysed by Friedman's repeated-measures ANOVA on ranks followed by Dunn's multiple-comparison to a single control point. Plasma catecholamine levels before and after hypercapnia/hypoxia were analysed by paired *t*-test (parametric data) or signed rank sum test (non-parametric data). All statistical tests, including determinations of normality and variance, were performed using commercial software (Sigmastat Windows). The fiducial limit of significance was set at 5 %.

Results

Hypercapnia

Dogfish

Respiratory acidosis was induced rapidly after the

Table 1. *Blood respiratory variables in dogfish (*Squalus acanthias*) and trout (*Oncorhynchus mykiss*) during hypercapnia and after an injection of catecholamines while hypercapnia was maintained*

	Dogfish (6)			Trout (5)		
	Pre-hypercapnia	Hypercapnia	Catecholamine injection	Pre-hypercapnia	Hypercapnia	Catecholamine injection
Ca_{O_2} (ml 100 ml^{-1})	3.87±0.36	3.63±0.35	3.73±0.35	7.24±0.81	6.20±1.23	7.89±1.02†
[Hb] (g 100 ml^{-1})	3.03±0.25	3.14±0.35	3.20±0.41	6.51±0.79	6.66±0.75	6.82±0.79
Haematocrit (%)	14.8±1.0	14.5±1.0	–	18.2±2.3	19.1±2.6	20.1±2.8
MCHC (g ml^{-1})	0.208±0.02	0.225±0.01	–	0.359±0.01	0.355±0.01	0.327±0.01
$[Ca_{O_2}]$/[Hb] (ml g^{-1})	1.24±0.08	1.15±0.06	1.17±0.08	1.14±0.10	0.88±0.12	1.15±0.03†

Data are means ± 1 S.E.M. There were no significant differences from pre-hypercapnia values; † indicates a significant difference (P<0.05) from the hypercapnia values.

MCHC, mean cellular haemoglobin concentration; Hb, haemoglobin.

N is indicated in parentheses.

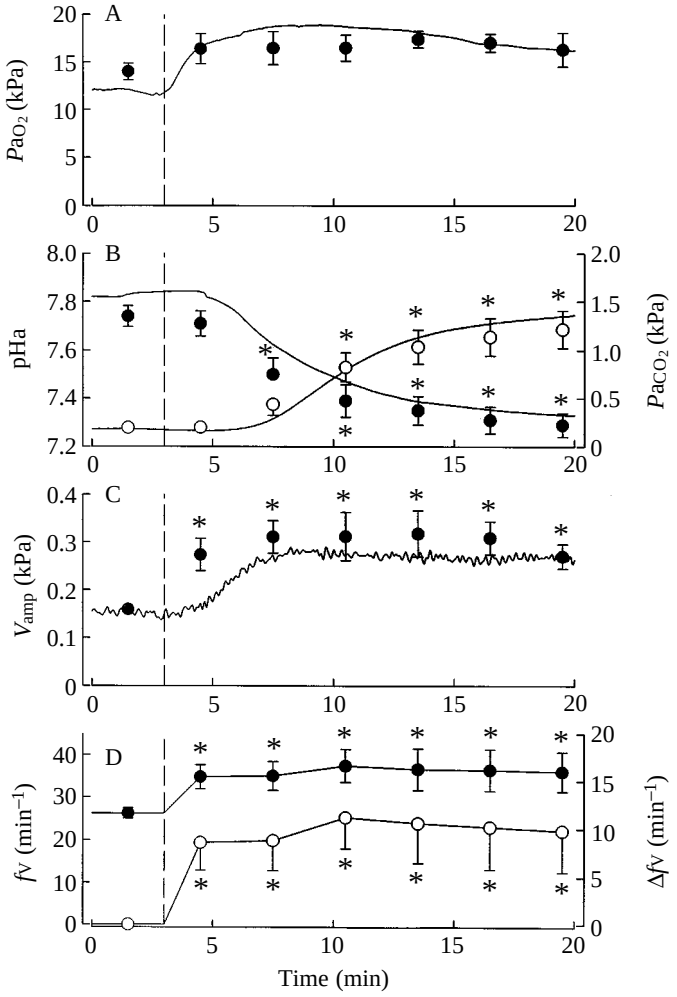

Fig. 1. The effects of acute hypercapnia in the dogfish (*Squalus acanthias*) on the continuously measured arterial blood and ventilatory variables (A) $P_{a_{O_2}}$, (B) pHa (filled symbols) and $P_{a_{CO_2}}$ (open symbols), (C) ventilation amplitude (V_{amp}) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish, whereas the symbols represent the mean data ± 1 S.E.M. (N=6). The beginning of the hypercapnic period is indicated by the dashed vertical lines. An asterisk indicates a statistical difference (P<0.05) from the pre-hypercapnia value.

commencement of hypercapnia as indicated by the concomitant depression of pHa and elevation of $P_{a_{CO_2}}$ (Fig. 1B). Ventilation amplitude (V_{amp}; Fig. 1C) and frequency (f_V; Fig. 1D) were increased within 1.5 min of initiating hypercapnia. After the first 7.5 min of hypercapnia, V_{amp} had re-stabilised at a level that was approximately 100 % greater than the pre-hypercapnia level. The increases in f_V mirrored the changes in V_{amp} and after 7.5 min f_V had increased by approximately 10 min^{-1}. Despite the hyperventilation, $P_{a_{O_2}}$ did not increase significantly during hypercapnia, although there did appear to be a trend for elevated values (Fig. 1A).

Table 1 illustrates the effects of hypercapnia on $C_{a_{O_2}}$, [Hb], Hct, mean cellular Hb concentration (MCHC) and $C_{a_{O_2}}$/[Hb]; none of these variables was significantly altered during the hypercapnia exposure.

Table 2. *Plasma catecholamine levels during hypercapnia or hypoxia in trout (*Oncorhynchus mykiss*) and dogfish (*Squalus acanthias*)*

	[Adrenaline] (nmol l^{-1})	[Noradrenaline] (nmol l^{-1})	[Total catecholamine] (nmol l^{-1})
Dogfish			
Pre-hypercapnia (5)	4.4±1.6	8.6±7.5	12.9±8.2
Hypercapnia (5)	5.4±1.4	6.2±1.5	11.6±2.1
Pre-hypoxia (12)	3.0±1.1	3.4±0.8	6.4±1.9
Hypoxia (12)	6.4±4.7	7.1±1.9	13.4±6.2
Trout			
Pre-hypercapnia (6)	16.8±9.6	3.3±1.3	20.1±10.8
Hypercapnia (6)	80.6±27.9*	5.4±1.1	86.0±28.8*
Pre-hypoxia (6)	10.0±5.4	4.0±0.8	14.0±5.0
Hypoxia (6)	150.3±44.9*	22.6±4.4*	172.9±45.8*

Data are means ± 1 S.E.M.; * indicates a significant difference (P<0.05) from the pre-exposure values.
N is indicated in parentheses.

Plasma catecholamine levels were unaffected by acute hypercapnia in dogfish (Table 2). The total catecholamine (adrenaline plus noradrenaline) concentrations were 12.9±8.2 and 11.6±2.1 nmol l^{-1} before and during hypercapnia, respectively.

The intra-arterial injection of a catecholamine cocktail, formulated to produce peak levels in the circulation of 125 nmol l^{-1} adrenaline and 125 nmol l^{-1} noradrenaline, was without effect on $P_{a_{O_2}}$, pHa, $P_{a_{CO_2}}$ and V_{amp} (Fig. 2A–C). However, there was a significant reduction in f_V of approximately 3–4 min^{-1} after 1.5 min (Fig. 2D). The catecholamine injection also did not affect $C_{a_{O_2}}$, [Hb] or $C_{a_{O_2}}$/[Hb] (Table 1).

Rainbow trout

The respiratory acidosis and the increase in V_{amp} associated with hypercapnia (Fig. 3) were similar to those observed in dogfish. V_{amp} reached a plateau after 10.5 min of hypercapnia, at which time it had increased by approximately 260 % over pre-hypercapnia values (Fig. 3C). Although there was a tendency for increased f_V during hypercapnia, the variability of the data was high and the changes were not statistically significant (Fig. 3D). Unlike in the dogfish, there was a significant increase in $P_{a_{O_2}}$ during the period of hypercapnia (Fig. 3A).

As in dogfish, $C_{a_{O_2}}$, [Hb], Hct, MCHC and $C_{a_{O_2}}$/[Hb] were unchanged after 20 min of hypercapnia (Table 1).

The circulating levels of total catecholamines (Table 2) were increased markedly during hypercapnia *solely* as a result of increased levels of adrenaline (16.8±9.6 and 80.6±27.9 nmol l^{-1} before and during hypercapnia, respectively); noradrenaline levels remained unchanged during hypercapnia.

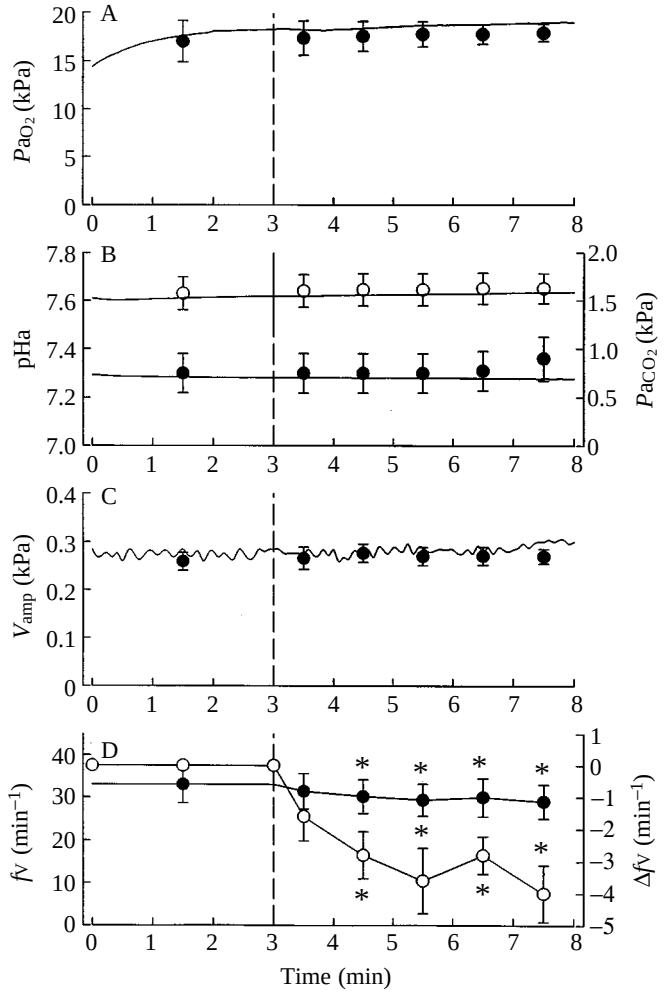

Fig. 2. The effects of an intra-arterial injection of a catecholamine cocktail (estimated peak circulating levels 125 nmol l^{-1} adrenaline, 125 nmol l^{-1} noradrenaline) during hypercapnia in the dogfish (*Squalus acanthias*) on the continuously measured arterial blood and ventilatory variables (A) P_{aO_2}, (B) pHa (filled symbols) and P_{aCO_2} (open symbols), (C) ventilation amplitude (V_{amp}) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish, whereas the symbols represent the mean data ± 1 S.E.M. (N=6). The catecholamine injection is indicated by the dashed vertical lines. An asterisk indicates a statistical difference (P<0.05) from the pre-injection value.

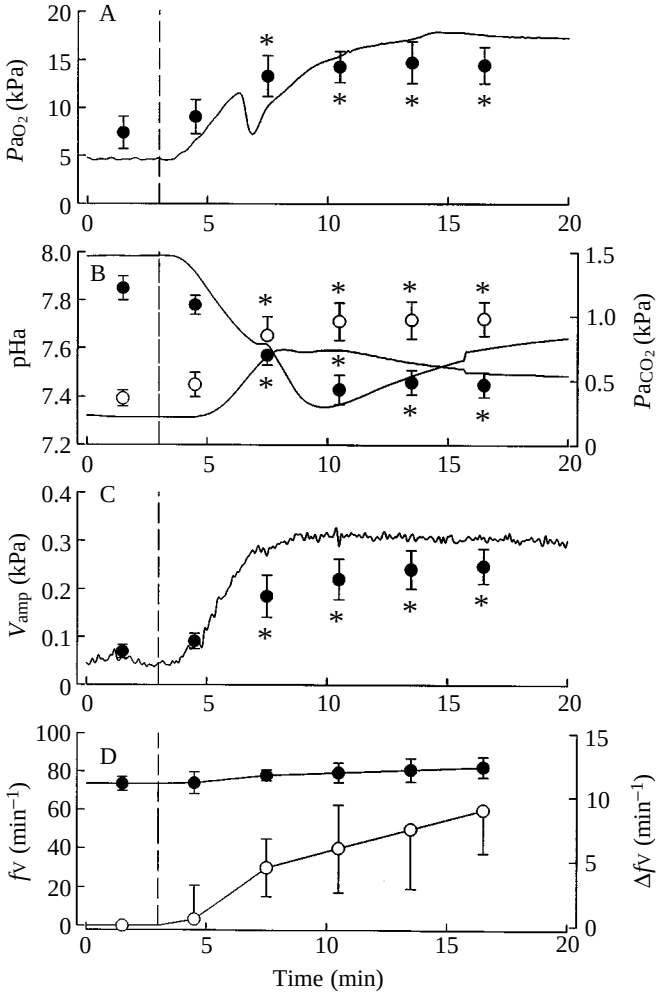

Fig. 3. The effects of acute hypercapnia in the rainbow trout (*Oncorhynchus mykiss*) on the continuously measured arterial blood and ventilatory variables (A) P_{aO_2}, (B) pHa (filled symbols) and P_{aCO_2} (open symbols), (C) ventilation amplitude (V_{amp}) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish, whereas the symbols represent the mean data ± 1 S.E.M. (N=5). The beginning of the hypercapnic period is indicated by the vertical dashed lines. An asterisk indicates a statistical difference (P<0.05) from the pre-hypercapnia value.

The intra-arterial injection of a catecholamine cocktail, formulated to produce peak levels in the circulation of 200 nmol^{-1} adrenaline and 50 nmol l^{-1} noradrenaline, activated rbc β-adrenergic Na^+/H^+ exchange as indicated by the resultant sudden metabolic acidosis (Fiévet *et al.* 1987; Fig. 4B). Concurrently, there was a pronounced reduction in V_{amp} after 1 min that persisted for the duration of the observation period (Fig. 4C); f_V was unaffected (Fig. 4D).

The catecholamine injection during hypercapnia in trout elicited significant increases in C_{aO_2} and O_2 specifically bound to haemoglobin (C_{aO_2}/[Hb], Table 1); [Hb], Hct and MCHC were not significantly affected.

Hypoxia

Dogfish

The exposure of dogfish to acute external hypoxia caused a progressive decline in P_{aO_2}; a minimal mean value of 2.0 kPa was attained after 30 min (Fig. 5A). The hypoxia was associated with an obvious respiratory alkalosis (increased pHa, decreased P_{aCO_2}; Fig. 5B) and pronounced increases in both V_{amp} (Fig. 5C) and f_V (Fig. 5D).

After 30 min of hypoxia, C_{aO_2} and C_{aO_2}/[Hb] were lowered by 61 % and 57 %, respectively (Table 3); [Hb], Hct and MCHC were unaffected.

The effects of hypoxia on plasma catecholamine levels in

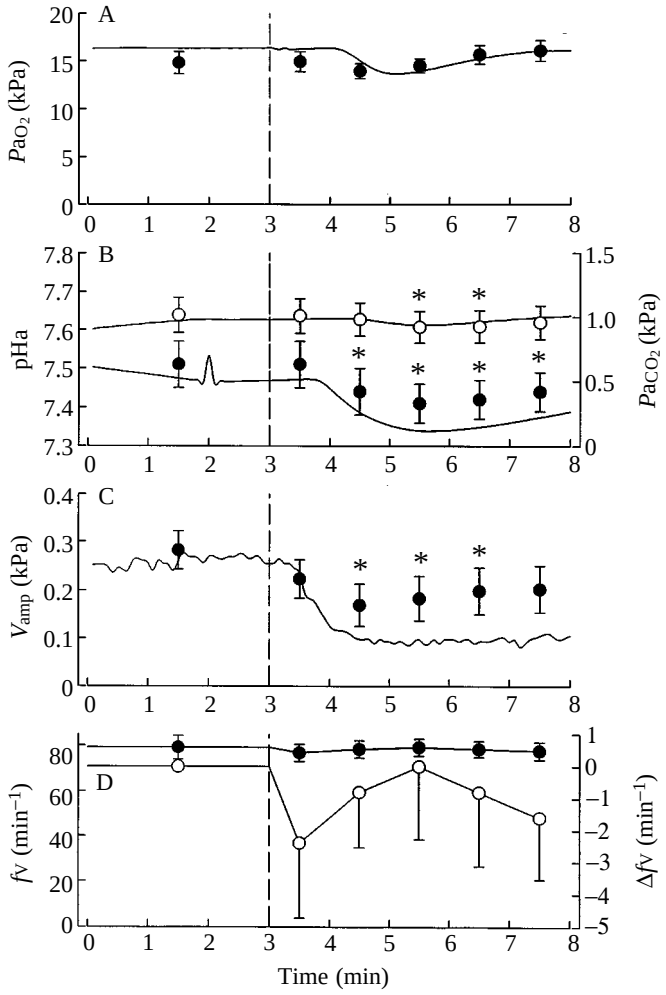

Fig. 4. The effects of an intra-arterial injection of a catecholamine cocktail (estimated peak circulating levels 200 nmol l^{-1} adrenaline, 50 nmol l^{-1} noradrenaline) during hypercapnia in the rainbow trout (*Oncorhynchus mykiss*) on the continuously measured arterial blood and ventilatory variables (A) $P_{a_{O_2}}$, (B) pHa (filled symbols) and $P_{a_{CO_2}}$ (open symbols), (C) ventilation amplitude (V_{amp}) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish, whereas the symbols represent the mean data ± 1 S.E.M. (N=5). The catecholamine injection is indicated by the vertical dashed lines. An asterisk indicates a statistical difference (P<0.05) from the pre-injection value.

dogfish were highly variable and thus, despite the relatively large number of fish used in this experiment (N=12), the concentrations were not statistically elevated after 30 min (Table 2). For each fish, additional serial blood samples were removed during the progressive hypoxia and analysed for plasma catecholamine levels only; these data, compiled with the ventilation results, are shown in Fig. 6A. This type of presentation clearly shows the absence of any pronounced elevation of catecholamine levels during hypoxia and, moreover, demonstrates the lack of any correspondence between plasma catecholamine levels and V_{amp}. Fig. 5C illustrates the relationship between plasma catecholamine levels and V_{amp} in an individual representative fish. It clearly shows that the hyperventilatory response commenced in the absence of any elevation in catecholamine levels. Although this particular fish did appear to release catecholamines into the circulation later during the hypoxia exposure (Fig. 5C), there was no noticeable impact on V_{amp}.

The injection of catecholamines into hypoxic dogfish was without effect on any measured variable (Fig. 7; Table 3), except for a significant decrease in f_V of 3–4 min^{-1} (Fig. 7D).

Rainbow trout

Exposure of rainbow trout to hypoxia caused a similar, although slightly less severe, reduction in $P_{a_{O_2}}$ (Fig. 8A) to that in the dogfish. V_{amp} increased progressively during hypoxia, reaching a new plateau after approximately 20 min (Fig. 8C). Unlike in the dogfish, there was no statistically significant increase in f_V (Fig. 8D). Owing to the hyperventilation, $P_{a_{CO_2}}$ declined during hypoxia yet pHa did not increase significantly. As in the dogfish, hypoxia was associated with marked reductions of $C_{a_{O_2}}$ and $C_{a_{O_2}}$/[Hb] of

Table 3. *Blood respiratory variables in dogfish (*Squalus acanthias*) and trout (*Oncorhynchus mykiss*) during hypoxia and after an injection of catecholamines while hypoxia was maintained*

	Dogfish (12)			Trout (6)		
	Pre-hypoxia	Hypoxia	Catecholamine injection	Pre-hypoxia	Hypoxia	Catecholamine injection
$C_{a_{O_2}}$ (ml 100 ml^{-1})	3.54±0.32	1.37±0.09*	1.50±0.26*	7.71±1.67	3.24±0.77*	3.57±0.60*
[Haemoglobin] (g 100 ml^{-1})	2.91±0.20	2.81±0.21	3.08±0.30	6.17±1.06	5.91±0.89	5.82±0.88
Haematocrit (%)	14.3±1.6	14.4±1.6	15.6±1.8	18.5±3.5	18.2±3.3	19.9±3.8
MCHC (g ml^{-1})	0.232±0.02	0.241±0.02	0.228±0.02	0.342±0.02	0.339±0.02	0.309±0.02*,†
[$C_{a_{O_2}}$]/[Hb] (ml g^{-1})	1.14±0.11	0.49±0.02*	0.47±0.06*	1.21±0.08	0.53±0.06*	0.65±0.08*,†

Data shown are means ± 1 S.E.M.; * indicates a significant difference (P<0.05) from the pre-hypoxia values; † indicates a significant difference (P<0.05) from the hypoxia values.

MCHC, mean cellular haemoglobin concentration; Hb, haemoglobin.

N is indicated in parentheses.

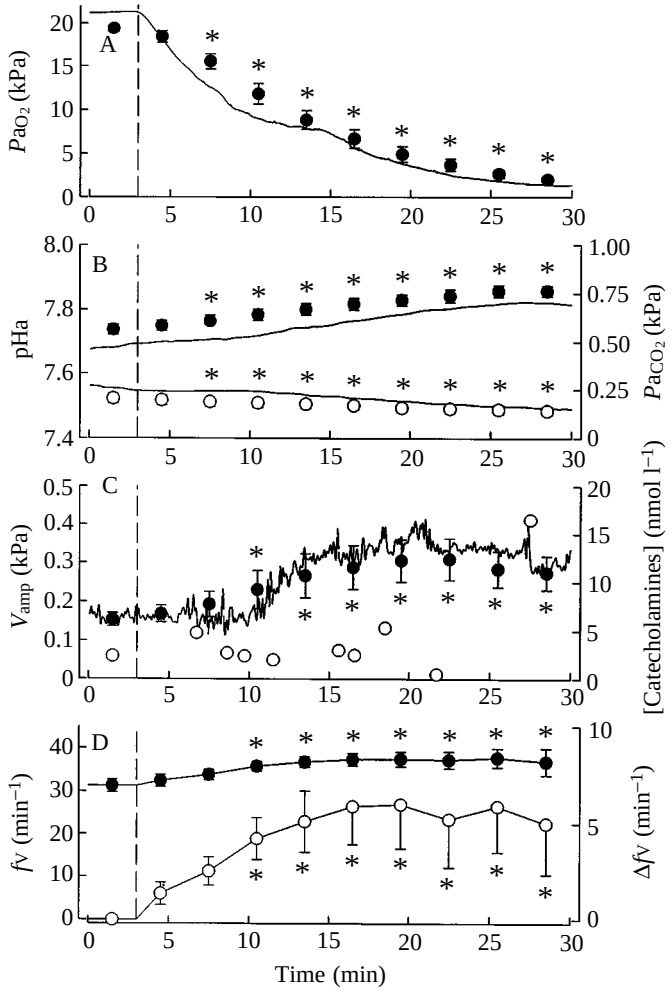

Fig. 5. The effects of acute hypoxia in the dogfish (*Squalus acanthias*) on the continuously measured arterial blood and ventilatory variables (A) $P_{a_{O_2}}$, (B) pHa (filled symbols) and $P_{a_{CO_2}}$ (open symbols), (C) ventilation amplitude (V_{amp}) (filled symbols) and plasma catecholamine levels ([Catecholamines]; open symbols) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish; the plasma catecholamine data for this same fish are shown in C. All other symbols represent the mean data ± 1 S.E.M. (N=12). The beginning of the hypoxic period is indicated by the vertical dashed lines. An asterisk indicates a statistical difference (P<0.05) from the pre-hypoxia value.

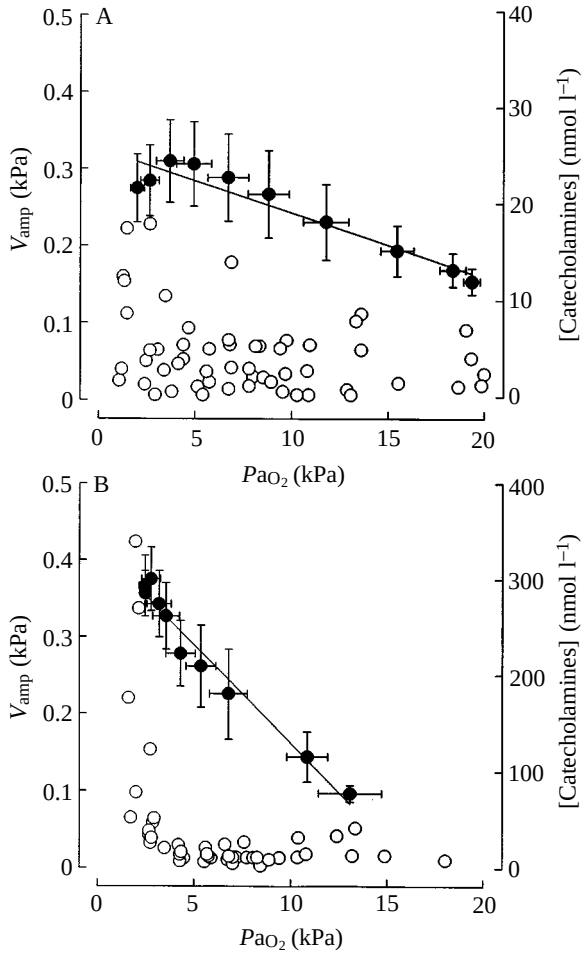

Fig. 6. The relationship between $P_{a_{O_2}}$ and ventilation amplitude (V_{amp}; filled symbols) and plasma total catecholamine (adrenaline plus noradrenaline) levels ([Catecholamines]; open symbols) during progressive hypoxia in (A) the dogfish (*Squalus acanthias*; N=12) and (B) the rainbow trout (*Oncorhynchus mykiss*; N=6). The ventilation and $P_{a_{O_2}}$ data are shown as mean values ± 1 S.E.M., whereas the catecholamine data are shown as a series of single points obtained by serial blood sampling of the same group of fish. In dogfish, the regression equation relating $P_{a_{O_2}}$ and V_{amp} is: V_{amp}=–0.011$P_{a_{O_2}}$+3.32; r^2=0.90; in trout, the regression equation is: V_{amp}=–0.035$P_{a_{O_2}}$+4.26; r^2=0.97.

58 % and 56 %, respectively; [Hb], Hct and MCHC were unaffected (Table 3).

Hypoxia caused a marked release of catecholamines into the circulation, with adrenaline being the predominant catecholamine secreted (Table 2). Fig. 8 illustrates the dynamics and consequences of catecholamine release during hypoxia in an individual representative fish. In this particular fish, there was a brief period of agitation which preceded an abrupt release of catecholamines (Fig. 8C) as $P_{a_{O_2}}$ reached approximately 2.7 kPa (Fig. 8A). This release of catecholamines elicited β-adrenergic activation of the rbc Na^+/H^+ exchanger, as indicated by the concurrent sudden metabolic acidosis. There was no obvious change in the ventilatory response of this fish upon elevation of catecholamine levels (Fig. 8C). The absence of any impact of catecholamine release on V_{amp} in rainbow trout during hypoxia is indicated further by Fig. 6B. This presentation clearly shows that catecholamines were released into the circulation at a $P_{a_{O_2}}$ threshold of approximately 2.7 kPa, with no obvious impact on V_{amp}, and that the hyperventilatory response to hypoxia occurred in the absence of elevated circulating catecholamine levels.

The injection of catecholamines into the arterial circulation of trout during hypoxia activated rbc Na^+/H^+ exchange as shown by the consequent metabolic acidosis (Fig. 9C).

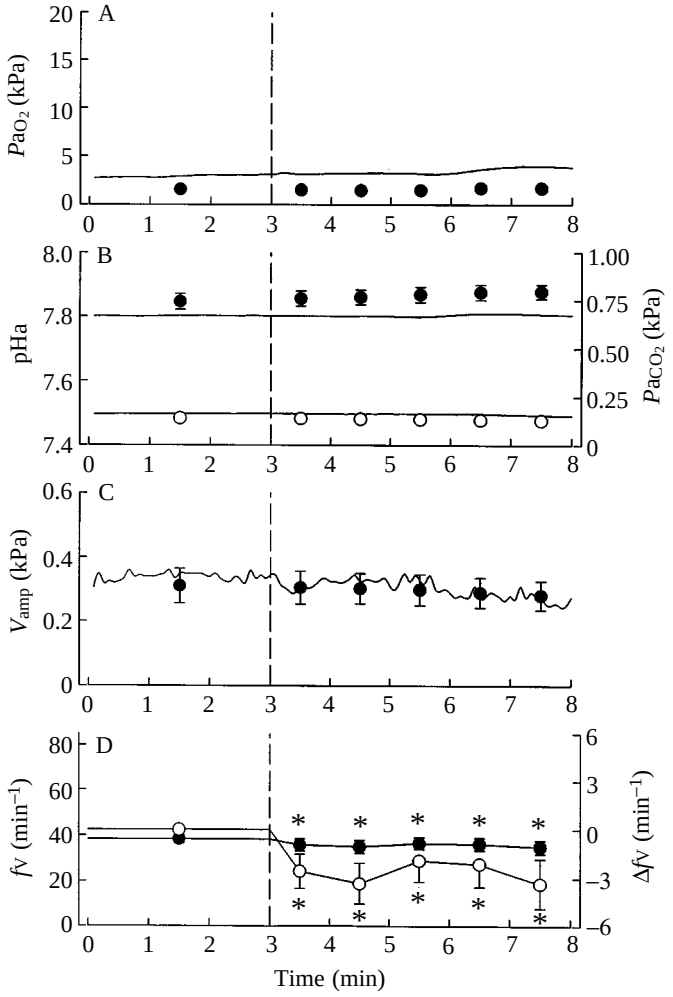

Fig. 7. The effects of an intra-arterial injection of a catecholamine cocktail (estimated peak circulating levels 125 nmol l^{-1} adrenaline, 125 nmol l^{-1} noradrenaline) during hypoxia in the dogfish (*Squalus acanthias*) on the continuously measured arterial blood and ventilatory variables (A) P_{aO_2}, (B) pHa (filled symbols) and P_{aCO_2} (open symbols), (C) ventilation amplitude (V_{amp}) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish, whereas the symbols represent the mean data ± 1 S.E.M. (N=9). The catecholamine injection is indicated by the vertical dashed lines. An asterisk indicates a statistical difference (P<0.05) from the pre-injection value.

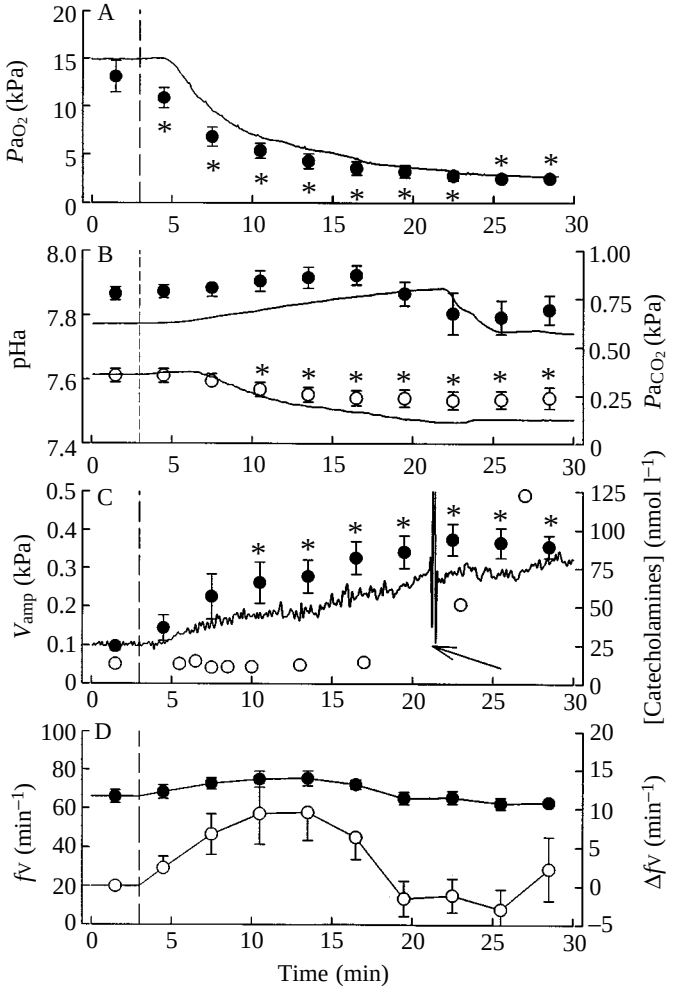

Fig. 8. The effects of acute hypoxia in the rainbow trout (*Oncorhynchus mykiss*) on the continuously measured arterial blood and ventilatory variables (A) P_{aO_2}, (B) pHa (filled symbols) and P_{aCO_2} (open symbols), (C) ventilation amplitude (V_{amp}) (filled symbols) and plasma catecholamine levels ([Catecholamines]; open symbols) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish; the plasma catecholamine data for this same fish are shown in C. The arrow indicates a brief period of agitation by this fish (see text for details). All other symbols represent the mean data ± 1 S.E.M. (N=6). The beginning of the hypoxic period is indicated by the vertical dashed lines. An asterisk indicates a statistical difference (P<0.05) from the pre-hypoxia value.

Activation of the rbc Na^+/H^+ exchanger was associated with a rise in C_{aO_2}/[Hb] and a reduction in MCHC (most likely related to cell swelling; Table 3). However, both V_{amp} and f_V were unaffected (Fig. 9C,D).

Discussion

Plasma catecholamine levels during hypercapnia and hypoxia

This is the first study to report plasma catecholamine levels in an elasmobranch during hypercapnia and only the second study to evaluate the effects of *acute* hypoxia on elasmobranch plasma catecholamine levels. Several previous studies have demonstrated an elevation of plasma catecholamine levels during stress (physical disturbance, exercise, hypoxia) in dogfish species (Butler *et al.* 1978, 1979, 1986; Opdyke *et al.* 1982; Metcalfe and Butler, 1989). In the present study, however, the Pacific spiny dogfish (*Squalus acanthias*) failed to secrete significant quantities of either adrenaline or noradrenaline into the circulation under similar, or even more severe, conditions of hypercapnia or hypoxia to those that

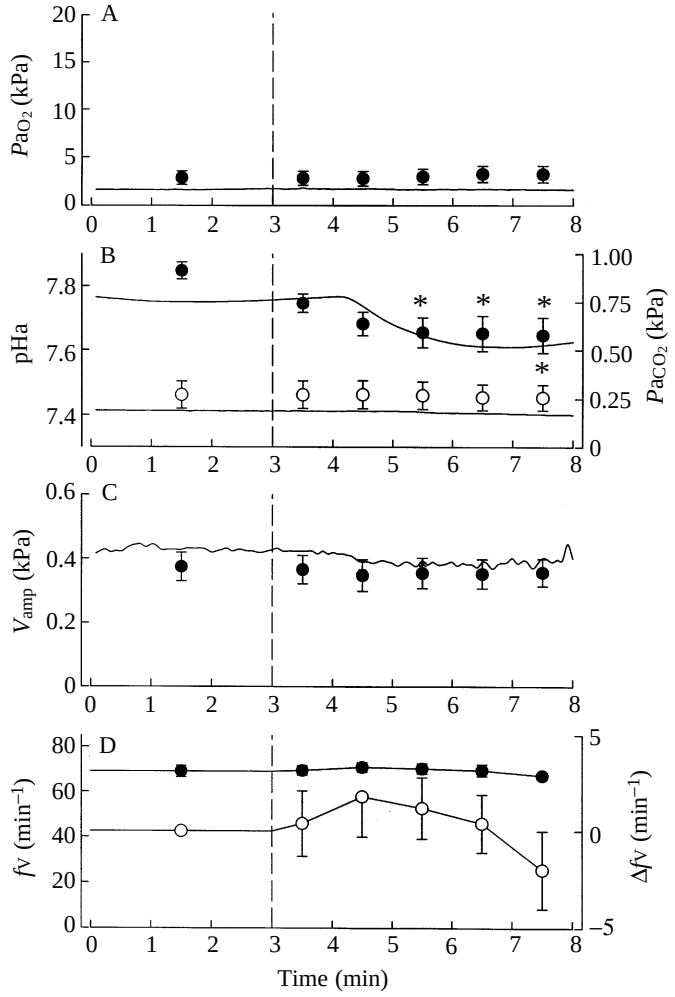

Fig. 9. The effects of an intra-arterial injection of a catecholamine cocktail (estimated peak circulating levels 200 nmol l^{-1} adrenaline, 50 nmol l^{-1} noradrenaline) during hypoxia in the rainbow trout (*Oncorhynchus mykiss*) on the continuously measured arterial blood and ventilatory variables (A) P_{aO_2}, (B) pHa (filled symbols) and P_{aCO_2} (open symbols), (C) ventilation amplitude (V_{amp}) and (D) ventilation frequency (f_V) (filled symbols) and the change in f_V (Δf_V; open symbols). The solid lines in A–C illustrate continuously recorded data from a single representative fish, whereas the symbols represent the mean data ± 1 S.E.M. (N=6). The catecholamine injection is indicated by the vertical dashed lines. An asterisk indicates a statistical difference (P<0.05) from the pre-injection value.

elicited pronounced catecholamine release in the rainbow trout (*Oncorhynchus mykiss*).

Hypercapnia

This and previous studies (Perry *et al.* 1987, 1989; Thomas *et al.* 1994) have shown hypercapnia to be a potent stimulus for catecholamine release in the rainbow trout. It has been demonstrated, however, that the specific stimulus promoting catecholamine release under such conditions is not respiratory acidosis *per se*, but rather the hypoxaemia induced by an associated Root effect (Perry *et al.* 1989). Elasmobranchs lack a Root effect (see Butler and Metcalfe, 1988), and thus the absence of catecholamine release in the dogfish during acute hypercapnia further supports the hypothesis that acidosis *in itself* is not a specific stimulus for catecholamine release in fish. This need not imply, however, that catecholamine release is controlled solely by blood oxygen status in fish, and indeed the results of the present hypoxia experiments do not support a close coupling between blood oxygen status and plasma catecholamine levels in the dogfish (see below).

Hypoxia

The impact of hypoxia on catecholamine release in fish has been addressed predominantly using teleost species (usually rainbow trout) as model systems (see Fig. 3 in Randall and Perry, 1992; Table 2 in Gamperl *et al.* 1994). Although a potent stimulus, it is apparent that the degree of hypoxia must be severe before catecholamine release is elicited in trout (e.g. Fiévet *et al.* 1987, 1990; Ristori and Laurent, 1989). Indeed, it has been shown experimentally that catecholamine release during external hypoxia in American eels (*Anguilla rostrata*) and trout is triggered at specific P_{aO_2} thresholds corresponding to approximately 50–60 % Hb O_2-saturation (Perry and Reid, 1992, 1994; Thomas and Perry, 1992; Thomas *et al.* 1992). The notion of a specific P_{aO_2} threshold for catecholamine release in trout during hypoxia is supported by the results of the present study in which an abrupt elevation occurred as P_{aO_2} decreased below 2.7 kPa (Fig. 6B). The physiological significance of such a precise coupling between blood oxygen status and catecholamine release in trout presumably arises from the beneficial effects of circulating catecholamines on blood oxygen transport, an effect largely mediated by β-adrenergic activation of rbc Na^+/H^+ exchange (reviewed by Thomas and Motais, 1990; Fiévet and Motais, 1991; Nikinmaa, 1992; Thomas and Perry, 1992). Briefly, the activation of the rbc Na^+/H^+ exchanger serves to enhance Hb O_2-binding owing to the concerted effects of increased rbc pHi and reduced concentrations of intracellular organic phosphates.

Dogfish lack a β-adrenergic rbc Na^+/H^+ exchanger (Tufts and Randall, 1989; Wood *et al.* 1994), and thus the need to link catecholamine release specifically to a precise blood O_2 status threshold in these fish is questionable. However, dogfish lack sympathetic innervation of the heart and systemic vasculature (Nilsson, 1983) and thus might be expected to release catecholamines earlier (at higher P_{aO_2} values) than trout during progressive hypoxia. Furthermore, it has been suggested that hyperventilation in dogfish is mediated *exclusively* by circulating catecholamines (Randall and Taylor, 1991) and, if so, catecholamine release at milder levels of hypoxia might be expected. The results of the present study, however, demonstrated no appreciable catecholamine release in the spiny dogfish during progressive hypoxia, despite reductions in C_{aO_2} that were even more severe than in the rainbow trout.

Although, in the present study, hypoxia did not elicit catecholamine release in the Pacific spiny dogfish (*Squalus acanthias*), previous studies utilising the lesser spotted dogfish (*Scyliorhinus canicula*) did reveal significant catecholamine

release after acute (1.5 h) or chronic (16–72 h) exposures (Butler *et al.* 1978, 1979; Metcalfe and Butler, 1989). The differences between this and previous studies may reflect species differences or differences in the duration of the hypoxic exposure. It is unlikely that the lack of catecholamine release in the present study was attributable to the hypoxia not being sufficiently severe, because the reduction in $P_{a_{O_2}}$ was greater than in the previous studies. Regardless of the reasons for the discrepancies, the absence of catecholamine release in spiny dogfish during hypoxia casts doubt on the physiological role for these hormones in ventilatory control and regulation of blood O_2 transport (see below).

Ventilatory and respiratory responses to hypercapnia and hypoxia

Dogfish

Relatively few studies have examined the effects of hypercapnia on ventilation in elasmobranchs (Randall *et al.* 1976; Wood *et al.* 1990; Graham *et al.* 1990). Those studies and the current results, however, clearly reveal that respiratory acidosis (or an associated variable), in itself, is a potent ventilatory stimulant, given the lack of an effect of hypercapnia on blood oxygen status in these fish. A similar conclusion was reached by Heisler *et al.* (1988), who reported a significant correlation between $P_{a_{CO_2}}$ and ventilation during hyperoxia in *Scyliorhinus stellaris*. Thus, while the role of CO_2/acidosis as a ventilatory stimulant in fish is often discounted (Randall, 1982, 1990; Shelton *et al.* 1986), there is abundant evidence to indicate its importance in both elasmobranchs and teleosts (see below).

The ventilatory response of elasmobranchs to hypoxia has been studied in detail, yet exceedingly variable results have been reported (Satchell, 1961; Piiper *et al.* 1970; Butler and Taylor, 1971, 1975; Short *et al.* 1979; Metcalfe and Butler, 1988). It has been suggested that the variable responses of dogfish to hypoxia may reflect their sensitivity to operative and/or confinement stress (Butler and Metcalfe, 1988). For example, dogfish exhibiting low initial f_V values are more likely to experience an increased f_V in response to hypoxia (Butler and Metcalfe, 1988). The dogfish used in the present study did not exhibit a high initial f_V (e.g. see Metcalfe and Butler, 1984) and this may explain, in part, the pronounced increases in both f_V and V_{amp} during progressive hypoxia. The results of the present study do not support a general belief (e.g. see Butler and Metcalfe, 1988; Fritsche and Nilsson, 1992) that elasmobranchs display a blunted ventilatory response to hypoxia.

Rainbow trout

The ventilatory responses of trout to hypercapnia and hypoxia were similar to those reported previously (for reviews, see Randall, 1982, 1990; Perry and Wood, 1989; Fritsche and Nilsson, 1992). During both hypercapnia and hypoxia, the hyperventilatory response consisted solely of an increase in V_{amp}. This contrasted with the response of the dogfish, in which increases in V_{amp} and f_V contributed to the hyperventilatory response.

Although the principal ventilatory drive in teleosts is generally accepted to be O_2-related (e.g. see review by Randall, 1982), there is emerging evidence that CO_2 plays a specific, albeit supplementary, role (Kinkead and Perry, 1991; Wood and Munger, 1994; reviewed by Perry and Wood, 1989). In the present study, trout exhibited pronounced hyperventilation during hypercapnia yet blood O_2 status was unaffected. Given the improbability of catecholamines stimulating ventilation (see below) and the absence of other ventilatory stimulants, it seems likely that the hyperventilation was being driven by the respiratory acidosis.

Physiological consequences of elevated circulating catecholamine levels

Dogfish

The results of the present study clearly do not support a role for circulating catecholamines in stimulating ventilation or modifying gas transfer or blood O_2 transport during hypercapnia or hypoxia in the Pacific spiny dogfish. First, catecholamines were not released into the circulation under either condition and the hyperventilatory responses occurred despite the absence of catecholamine release. Second, the injection of catecholamines into the circulation during hypercapnia or hypoxia, to mimic the levels of adrenaline and noradrenaline that were measured in previous studies on a related dogfish, *Scyliorhinus canicula*, failed to elicit hyperventilation or to modify any of the measured blood respiratory variables. Indeed, f_V was reduced significantly after catecholamine injections during both hypercapnia and hypoxia and, because V_{amp} was unaffected, it is likely that minute ventilation volume was decreased.

The only other study to address the influence of exogenous catecholamines on ventilation in dogfish (Taylor and Wilson, 1989) examined f_V alone. Moreover, that study was performed using a single catecholamine (adrenaline) under normocapnic normoxic conditions and thus it may not have adequately simulated the natural conditions of catecholamine release. In addition, only a small subset of the fish (three individuals) that were examined, displaying low *initial* f_V, experienced obvious increases in f_V after adrenaline injection. It was suggested that the ventilatory response to catecholamines was dependent upon the initial f_V and that fish already displaying an increased f_V would be unable to increase f_V further after catecholamine injection. Nevertheless, these data formed the basis of a hypothesis (see Randall and Taylor, 1991) in which hyperventilation in dogfish is driven solely by circulating catecholamine levels. The present results and those of previous studies, utilising either α-methyl-*p*-tyrosine to reduce catecholamine release (Metcalfe and Butler, 1989) or adrenergic receptor antagonists to prevent their actions (Metcalfe and Butler, 1988) during hypoxia, do not support this view. Indeed, the results of the present study indicate that natural catecholamine release under conditions of hypercapnia or hypoxia would be more likely to evoke hypoventilation than hyperventilation.

The injection of catecholamines into hypercapnic or hypoxic

dogfish did not affect any of the measured blood respiratory variables and thus there does not appear to be any obvious benefit to blood O_2 transport associated with an elevation of circulating catecholamine levels. Metcalfe and Butler (1988, 1989) reached a similar conclusion concerning the role of catecholamines in gas exchange during hypoxia in dogfish. Thus, the physiological significance of catecholamine release during stress in dogfish and other elasmobranchs has not yet been elucidated. Catecholamines are known to cause hyperglycaemia in dogfish (DeRoos and DeRoos, 1978), and thus it is possible that they have an important metabolic role in these fish. This may explain the occurrence of catecholamine release in hypoxic dogfish exposed for longer periods than in the present study (1.5–72 h; Butler *et al.* 1978, 1979; Metcalfe and Butler, 1989) during which a metabolic role may become more important.

Rainbow trout

The present results do not support a stimulatory role for catecholamines in the hyperventilatory response of trout to either hypercapnia or hypoxia, but instead indicate a potent hypoventilatory role during hypercapnia. Although catecholamines were released naturally during hypercapnia, it is improbable that this contributed to the hyperventilation because a further elevation after injection of exogenous catecholamines induced a pronounced hypoventilation (see also Kinkead and Perry, 1991; Perry *et al.* 1992). The hypoventilatory response occurred concurrently with activation of rbc Na^+/H^+ exchange and the resultant increase in C_{aO_2}. Thus, the catecholamine-mediated hypoventilation may have arisen secondarily as C_{aO_2} increased and relieved a portion of the O_2 ventilatory drive, which in trout appears to be related to C_{aO_2} (Smith and Jones, 1982; Randall, 1982). It has been argued (Perry *et al.* 1992) that a hypoventilatory response following catecholamine release accompanies a lowering of the specific ventilatory convection requirement and serves to lower the energetic costs associated with ventilation. The absence of a similar hypoventilatory response in dogfish (f_V decreased but V_{amp} remained constant) may reflect the absence of an rbc β-adrenergic Na^+/H^+ exchange system in these fish.

Under hypoxic conditions, catecholamine injections did not elicit hypoventilation, even though rbc Na^+/H^+ exchange was activated and C_{aO_2}/[Hb] was increased. It is possible that the relatively small effects on blood O_2 transport were insufficient to overcome the existing ventilatory drive arising from the hypoxaemia or that any small stimulatory effects of catecholamines on ventilation were offset by increased blood O_2 levels.

Although the results of studies employing catecholamine injections in fish have been cited to support a stimulatory role for these hormones in the control of breathing (see review by Randall and Taylor, 1991), it is apparent that these studies reveal a myriad of responses including no effect, hypoventilation or hyperventilation (Playle *et al.* 1990; Kinkead and Perry, 1990, 1991; Kinkead *et al.* 1991; Perry *et al.* 1992; Aota and Randall, 1993; Burleson and Milsom, 1995). It would appear, however, that hypoventilation (or no effect) is the more prevalent response, especially when using physiological levels of adrenaline (the predominant circulating catecholamine in teleosts). Of interest are two recent studies utilising rainbow trout (Aota and Randall, 1993; Burleson and Milsom, 1995) which reported exactly opposite effects of exogenous catecholamines on f_V and entirely different V_{amp} responses (Burleson and Milsom 1995; no effect of either catecholamine on V_{amp}; Aota and Randall, 1993; stimulatory effect on V_{amp} of noradrenaline only). Although Burleson and Milsom (1995) observed an increase in f_V after injecting high levels of either catecholamine, the physiological significance of this response is questionable considering that fish generally increase ventilation volume by large changes in V_{amp} and only small increases in f_V (Perry and Wood, 1989). Obvious hyperventilatory effects of exogenous catecholamines were demonstrated in the European eel (*Anguilla anguilla*; Peyreaud-Waitzenegger, 1979; Peyreaud-Waitzenegger *et al.* 1980), but the physiological relevance is also debatable because eels do not release catecholamines into their circulation during hypoxia except under extremely severe conditions (P_{wO_2}<4.7 kPa; Perry and Reid, 1992), whereas hyperventilation commences during much milder hypoxia (Peyreaud-Waitzenegger *et al.* 1980).

In summary, this study demonstrates convincingly that circulating catecholamines do not exert a stimulatory influence on ventilation in trout or dogfish during hypercapnia or hypoxia.

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Sadly, John Boom died in a tragic accident shortly after this research was completed. We dedicate this work to his memory.

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