DOES BLOOD ACID–BASE STATUS MODULATE CATECHOLAMINE SECRETION IN THE RAINBOW TROUT (ONCORHYNCHUS MYKISS)?

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Accepted 2 September; published on WWW 26 October 1998

Summary

The direct and modulating effects of acidosis on catecholamine secretion in rainbow trout (Oncorhynchus mykiss) were assessed in vivo using cannulated fish and in situ using a perfused cardinal vein preparation. In situ, acidosis (a reduction in perfusate pH from 7.9 to 7.4) did not elicit catecholamine release or modulate the secretion of catecholamines evoked by the non-specific cholinergic receptor agonist carbachol (3·10⁻⁷ to 10⁻⁵ mol kg⁻¹) or the muscarinic receptor agonist pilocarpine (10⁻⁷ mol kg⁻¹). Acidosis, however, significantly increased the secretion rates of noradrenaline and adrenaline in response to nicotine (10⁻⁸ to 10⁻⁷ mol kg⁻¹). In vivo, intra-arterial injections of nicotine (300–600 nmol kg⁻¹) into normocapnic or moderately hypercapnic fish (water P\(_{CO_2}\) =5 mmHg or 0.67 kPa) caused a dose-dependent elevation of circulating catecholamine levels. At the highest dose of nicotine, the rise in plasma catecholamine levels was significantly enhanced in the hypercapnic fish.

Acute hypoxia in vivo caused an abrupt release of catecholamines when arterial haemoglobin O\(_2\)-saturation was reduced to approximately 55–60 %; this catecholamine release threshold during hypoxia was unaltered in hypercapnic fish. However, the hypoxia-induced catecholamine release was significantly greater in hypercapnic fish than in normocapnic fish.

The results of this study suggest that blood acid–base status, while not influencing catecholamine secretion directly or influencing the blood O\(_2\) content threshold for catecholamine release during hypoxia, may modulate the secretory process specifically in response to nicotinic receptor stimulation of chromaffin cells.

Key words: adrenaline, noradrenaline, chromaffin cell, stress, hypoxia, hypercapnia, acidosis, catecholamine secretion, rainbow trout, Oncorhynchus mykiss.

Introduction

Adrenaline and noradrenaline are the primary catecholamines secreted by teleosts in response to severe acute stress (Randall and Perry, 1992; Wendelaar Bonga, 1997; Reid et al. 1998). Typical stressors include hypoxia (Boutilier et al. 1988; Ristori and Laurent, 1989; Fievet et al. 1990; Perry et al. 1991), hypercapnia (Perry et al. 1987, 1989), exposure to air (Walhqvist and Nilsson, 1980), physical disturbance (Nakano and Tomlinson, 1967; Ristori and Laurent, 1985) and exhaustive exercise (Pirimmet al. 1986). Upon release into the blood, catecholamines are involved in minimising the detrimental effects of acute stress on blood oxygen transport and are also believed to influence several other physiological processes (for reviews, see Perry and Wood, 1989; Thomas and Perry, 1992; Randall and Perry, 1992; Wendelaar Bonga, 1997).

In teleosts, the principal sources of circulating catecholamines are the chromaffin cells associated with the posterior cardinal vein and the anterior portion of the head kidney (Nandi, 1961; Nakano and Tomlinson, 1967; Nilsson, 1983). These cells are innervated by preganglionic nerve fibres of the sympathetic nervous system that release the neurotransmitter acetylcholine (Nilsson, 1983). Upon activation of cholinergic receptors (Reid and Perry, 1995; Al-Kharrat et al. 1997), chromaffin cells release catecholamines via exocytosis in a Ca\(^{2+}\)-dependent process (Furimsky et al. 1996).

Owing to the nature of the stressors that elicit catecholamine release in fish (see above), the chemical composition of the blood is generally profoundly altered during the secretory event. In particular, the blood is usually hypoxic and/or acidotic (see Randall and Perry, 1982). Only a few studies, however, have directly assessed the effects of altered blood oxygen status (Perry et al. 1991) or acid–base status (Dashow and Epplle, 1985; Perry et al. 1993) on catecholamine secretion in fish. Although blood acidosis does not directly stimulate catecholamine secretion in vivo in rainbow trout (Perry et al. 1989), there is indirect evidence to suggest that lowered pH may enhance the secretion of catecholamines during hypoxaemia (Thomas et al. 1994). To date, however, no studies have directly examined a possible role of blood acidosis in modulating catecholamine secretion during acute stress in fish.
The goals of this study, therefore, were to evaluate the short-term effects of acidosis on catecholamine secretion as well as to determine whether lowered blood pH (as might occur during acute stress) is able to modulate the response of chromaffin cells to cholinergic stimulation. The experiments were performed on rainbow trout in situ using a perfused posterior cardinal vein preparation (Fritsche et al. 1993) and in vivo using either serial blood-sampling techniques or an extracorporeal arterial blood shunt (Thomas, 1994).

Materials and methods

Experimental animals

Rainbow trout Oncorhynchus mykiss (Walbaum) of both sexes for in situ perfusion experiments (mass 290.6±9.5 g; mean ± s.e.m., N=116) were obtained from Thistle Springs Trout Farm. Additional fish for in vivo experiments (mean mass 825.1±35 g; N=12) were obtained from Linwood Acres Trout Farm. All fish were kept in large fibreglass tanks supplied with flowing, aerated and dechlorinated, city of Ottawa tap water (15 °C). Fish were maintained on a 12 h:12 h light:dark photoperiod and were fed ad libitum maintained normocapnic (control group). Hypercapnia was achieved by gassing a water equilibration column with 1.3 % CO₂ in air supplied by a Cameron gas-mixing flowmeter (model GF-3/MP) while the experimental saline was gassed with 1.0 % CO₂ in air supplied by a Wösthoff gas-mixing pump (model M301a/f). The control and experimental saline solutions were left to equilibrate for 3 h; the mean final pH values of the control and acidic salines were 7.9±0.04 and 7.4±0.02, respectively (means ± s.e.m.). The preparation was pre-perfused with control saline for 20 min using a positive pressure differences (approximately 15 cm difference between the surface of the saline and the cannula) to maintain a constant flow (1.5 ml min⁻¹) through the posterior cardinal vein. After 20 min, four pre-samples of perfusate were taken at 1 min intervals. Flow was then switched to a second beaker containing either control or acidic saline. For short-term perfusions, samples were collected for 10 min after the switch. For the longer-term perfusions, blood samples were collected at 5 min intervals for 30 min.

To determine whether acidosis was capable of modulating the effects of cholinergic stimulation on the chromaffin cells, preparations were perfused with either control (pH 7.9) or experimental (pH 7.4) saline. After the pre-treatment samples had been taken, a bolus injection of carbachol (carbamylcholine chloride; Sigma), nicotine (nicotine-di-D-tartrate; Sigma) or the muscarinic receptor agonist pilocarpine (hydrochloride; Sigma) was delivered through the inflow cannula into different preparations. The doses of carbachol used were 3×10⁻⁷, 10⁻⁶, 3×10⁻⁶ and 10⁻⁷ mol kg⁻¹ body mass; the doses of nicotine used were 10⁻⁸, 3×10⁻⁸ and 10⁻⁷ mol kg⁻¹; the dose of pilocarpine used was 10⁻⁷ mol kg⁻¹. The drugs were injected in 0.3 ml of saline. The doses administered are expressed as mol kg⁻¹ because the final concentration of agonist bathing the chromaffin cells cannot be determined. Samples were taken at 1 min intervals for 5 min.

In situ experiments

Fish were anaesthetised using 0.1 g l⁻¹ MS-222 adjusted to approximately pH 7.0 with 0.2 g l⁻¹ NaHCO₃. Fish were placed on a surgical table where their gills were irrigated continuously with anaesthetic solution. An indwelling polyethylene cannula (Clay-Adams, PE 50) was inserted into the dorsal aorta according to the method described by Soivio et al. (1975). The caudal vein and the caudal artery were cannulated at the level of the caudal peduncle using standard surgical procedures (see Axelsson and Fritsche, 1994). After surgery, the fish were placed into individual opaque acrylic boxes supplied with flowing, aerated fresh water, where they were allowed to recover for 24 h before experimentation.

Blood was monitored continuously for arterial O₂ tension (Pao₂), arterial CO₂ tension (Paco₂) and arterial pH (pHa) using an extracorporeal blood shunt (Thomas, 1994; Perry and Gilmour, 1996). A peristaltic pump (flow 0.4 ml min⁻¹) was used to withdraw blood from the caudal artery and return it to the caudal vein. The dorsal aortic cannula was used to monitor blood pressure and for blood sampling. analogue signals were converted to digital data and collected and stored on computer using a data-acquisition system (Biopac) and accompanying software (AcqKnowledge 3.0). Upon stabilisation of blood gas levels (usually within 15 min of starting the shunt), an initial blood sample (1 ml) was taken during normocapnic normoxia. The water supplying the fish box was then rendered hypercapnic (experimental group) or maintained normocapnic (control group). Hypercapnia was achieved by gassing a water equilibration column with 1.3 % CO₂ in air (Cameron flowmeter) to reach a final Paco₂ of approximately 6 mmHg (0.8 kPa). Previous studies have
shown that hypercapnia, if severe enough, can elicit catecholamine release in trout (see Randall and Perry, 1992). Thus, the levels of hypercapnia used in this experiment were selected on the basis of pilot experiments that showed stable plasma catecholamine levels during 20 min of exposure to 1.3% CO$_2$. After 20 min, a second blood sample was withdrawn. At this point, graded hypoxia was induced in both the control and experimental groups; this was accomplished by substituting N$_2$ for air. Blood samples were taken at 10 mmHg (1.3 kPa) ($P_{\text{aCO}_2}$) intervals until 35 mmHg (4.7 kPa) was reached, then every 5 mmHg (0.7 kPa) until catecholamine release was presumed to have occurred (indicators are struggling, a sudden lowering of pH$_a$ and a pronounced increase in blood pressure). The duration of the hypoxic exposures therefore varied among fish.

**In vivo nicotine experiments**

Dorsal aortic cannulae were placed into fish weighing between 200 and 450 g, as described above, and the fish were allowed to recover for 24 h before experimentation. Three blood samples (0.5 ml) were taken from each fish; an initial sample followed by samples at 2 and 5 min post-injection. Doses of nicotine of 300, 450 and 600 nmol kg$^{-1}$ (1 ml kg$^{-1}$) plus a saline injection (control) were administered to separate groups of fish. Experimental fish were exposed to 20 min of hypercapnia before sampling; %CO$_2$ and total flow from a Cameron flowmeter were adjusted to give a final $P_{\text{wCO}_2}$ of approximately 5 mmHg (0.7 kPa). Pilot experiments demonstrated that this level and duration of hypercapnia would not cause plasma catecholamine levels to increase.

**Analytical techniques**

In the extracorporeal shunt experiments, blood pH$_a$, $P_{\text{aCO}_2}$, and $P_{\text{aO}_2}$ were monitored using Radiometer (CO$_2$, O$_2$) and Metrohm (pH) electrodes housed in temperature-controlled cuvettes and connected to a Radiometer PHM 73 meter. $P_{\text{wCO}_2}$ and $P_{\text{wO}_2}$ were measured using additional Radiometer O$_2$ and CO$_2$ electrodes connected to a dual-channel O$_2$/CO$_2$ meter (Cameron Instruments). A continuous flow of water across the water-recording electrodes was achieved by siphon. The O$_2$ electrodes were calibrated by pumping (using the peristaltic pump of the extracorporeal shunt) a zero solution [2% (w/v) sodium sulphite] or air-saturated water continuously through the electrode sample compartments until stable readings were obtained. The CO$_2$ electrode was calibrated in a similar manner using mixtures of 0.5% and 1.0% CO$_2$ in air provided by a Cameron gas flowmeter. The pH electrode was calibrated using Radiometer precision buffers. The CO$_2$, O$_2$ and pH electrodes were calibrated prior to each experiment.

Blood pressure was monitored by connecting the dorsal aortic cannula to a pressure transducer (UFI model 1050 BP; UFI Morro Bay, CA, USA) linked to an amplifier (model MP100) and integrated with the data-acquisition system. The pressure transducer was calibrated daily against a static column of water.

**In vivo arterial blood samples** (40 μl) were analysed in triplicate for oxygen content ($C_{\text{aO}_2}$) using an Oxycon blood oxygen content analyser (Cameron Instruments). Total CO$_2$ ($C_{\text{aCO}_2}$) was analysed using true plasma (50 μl) with a Corning 965 carbon dioxide analyser. Haemoglobin concentration was determined in duplicate on 20 μl blood samples using a commercial spectrophotometric haemoglobin assay kit (Sigma).

All blood samples collected for measurements of catecholamines were centrifuged immediately (12000g for 1 min), and the plasma was placed in liquid N$_2$ and then stored at −80°C until subsequent analysis. Plasma samples were subjected to alumina extraction and then analysed by high-performance liquid chromatography (HPLC) with electrochemical detection (Woodward, 1982). 3,4-Dihydroxybenzylamine was used as an internal reference standard in all analyses. Detection limits for adrenaline and noradrenaline were 0.1 nmol l$^{-1}$. Inter-assay variations for noradrenaline and adrenaline were 6 and 3%, respectively; intra-assay variations were 5 and 8% for noradrenaline and adrenaline, respectively.

**Construction of oxygen equilibrium curves**

Oxygen specifically bound to haemoglobin (Hb) (mol O$_2$ mol$^{-1}$ Hb) was calculated after subtraction of physically dissolved O$_2$ in the plasma; O$_2$ capacitance coefficients for human plasma were obtained from Boutilier et al. (1984). $[\text{O}_2]/[\text{Hb}]$ was plotted against $P_{\text{aO}_2}$ (pre-hypercapnia samples were excluded for the experimental fish), and a sigmoid curve was fitted to the data. 100% O$_2$ haemoglobin-saturation values were obtained from the normocapnic and hypercapnic curves, and this allowed O$_2$ equilibrium curves to be expressed as a function of percentage haemoglobin O$_2$-saturation. $P_{50}$ values ($P_{\text{aO}_2}$ at 50% haemoglobin O$_2$-saturation) were determined automatically during the curve-fitting procedure using a commercial graphics software package (SigmaPlot 4.0).

**Statistical analyses**

Data are presented as means ± the standard error of the mean (S.E.M.) unless stated otherwise. Wherever agonist drugs were administered, maximal secretion rates were computed by determining maximal post-injection secretion rates for individual fish and calculating the mean value for each dose. The maximal post-injection secretion rate is defined as the highest rate of catecholamine secretion occurring during any 1 min period after injection (maximal secretion rate always occurred within 3 min of injection). Data were analysed statistically using one-way analysis of variance (ANOVA) or ANOVA on ranks to test for differences within a treatment group. This was followed by Dunn’s comparison with control pre-treatment values or Dunn’s multiple comparisons. Student’s t-test was used to detect differences between treatments. All calculations were performed using SigmaStat software package setting the fiducial limit at 5%.
Results

In situ experiments

Effects of acidosis on catecholamine secretion

Lowering the perfusate pH from 7.9 to 7.4 was without effect on the secretion rates of adrenaline or noradrenaline during a 10 min period of perfusion (Fig. 1). Although the rate of catecholamine secretion appeared to decline with time in both the control and acidic groups, the changes were not statistically significant. Indeed, there were no significant differences observed within or between the two groups at any time during the perfusion period.

A longer-term (30 min) perfusion study was conducted to determine whether acidosis might cause a delayed effect on catecholamine secretion. The results demonstrated that acidosis had no significant effects on catecholamine secretion over a 30 min perfusion period (Table 1).

Effects of acidosis on cholinergic-agonist-evoked catecholamine secretion

Carbachol. Maximal catecholamine secretion rates are illustrated in Fig. 2. Both the control (pH 7.9) and experimental (pH 7.4) treatments evoked dose-dependent release of catecholamines upon bolus injection of the non-specific cholinoreceptor agonist carbachol. The highest doses ($3 \times 10^{-6}$ and $10^{-5}$ mol kg$^{-1}$ for noradrenaline; $10^{-6}$ to $10^{-5}$ mol kg$^{-1}$ for adrenaline) induced a significant release compared with pre-injection values. However, at any dose, carbachol-evoked catecholamine secretion rate was unaffected by perfusate acid–base status (Fig. 2).

Nicotine. Nicotine-evoked secretion rates for noradrenaline and adrenaline are depicted in Fig. 3. Control preparations (pH 7.9) did not exhibit significant changes in the rates of noradrenaline release at any of the three doses tested ($10^{-8}$ to $10^{-7}$ mol kg$^{-1}$); significant changes in the adrenaline secretion rate occurred only at the highest nicotine dose ($10^{-7}$ mol kg$^{-1}$).
Acid–base status and catecholamine secretion in trout

Acidotic preparations, however, displayed significant increases in the rate of noradrenaline release (compared with the pre-injection sample) and significantly increased secretion rates compared with the control group for doses of $10^{-8}$ and $3 \times 10^{-8}$ mol kg$^{-1}$. At the higher doses ($3 \times 10^{-8}$ and $10^{-7}$ mol kg$^{-1}$), the preparations perfused with acidic saline displayed significantly greater rates of adrenaline secretion (Fig. 3B).

Pilocarpine. Noradrenaline and adrenaline secretion rates after a bolus injection of the specific muscarinic receptor agonist pilocarpine are shown in Fig. 4. Pilocarpine, when added to the control (pH 7.9) and acidotic (pH 7.4) preparations, stimulated noradrenaline secretion (Fig. 4A) equally. Adrenaline secretion rate (Fig. 4B) was statistically elevated by pilocarpine only in the acidotic preparations; statistical significance in the control group was prevented by a high degree of variance in the data set.

Table 1. The effects of perfusate pH on the secretion rates of noradrenaline (NADR) and adrenaline (ADR) in situ following a switch from control saline (pH 7.9) to acidic saline (pH 7.4)

<table>
<thead>
<tr>
<th>Sample (min)</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NADR (pmol min$^{-1}$)</td>
<td>ADR (pmol min$^{-1}$)</td>
</tr>
<tr>
<td>Pre-acidosis</td>
<td>7.6±4.0</td>
<td>48.1±8.3</td>
</tr>
<tr>
<td>5</td>
<td>9.2±3.4</td>
<td>30.3±7.3</td>
</tr>
<tr>
<td>10</td>
<td>10.4±3.4</td>
<td>16.7±7.9</td>
</tr>
<tr>
<td>15</td>
<td>5.7±2.5</td>
<td>17.5±3.4</td>
</tr>
<tr>
<td>20</td>
<td>3.7±1.9</td>
<td>15.3±5.3</td>
</tr>
<tr>
<td>25</td>
<td>5.2±3.7</td>
<td>14.0±4.3</td>
</tr>
<tr>
<td>30</td>
<td>3.1±1.6</td>
<td>5.0±2.0</td>
</tr>
</tbody>
</table>

In the control group, pH was kept constant at 7.9 for the duration of the experiment.

Values are mean secretion rates ± 1 S.E.M., N=6 animals.

Acidic preparations, however, displayed significant increases in the rate of noradrenaline release (compared with the pre-injection sample) and significantly increased secretion rates compared with the control group for doses of $10^{-8}$ and $3 \times 10^{-8}$ mol kg$^{-1}$. At the higher doses ($3 \times 10^{-8}$ and $10^{-7}$ mol kg$^{-1}$), the preparations perfused with acidic saline displayed significantly greater rates of adrenaline secretion (Fig. 3B).

Table 2. The effects of short-term (20 min) hypercapnia on selected arterial blood respiratory variables in rainbow trout

<table>
<thead>
<tr>
<th>Hypercapnic group</th>
<th>Pre</th>
<th>20min</th>
<th>Control</th>
<th>Pre</th>
<th>20min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_aCO_2$ (mmHg)</td>
<td>2.08±0.09</td>
<td>5.83±0.44$^\dagger$</td>
<td>2.64±0.41</td>
<td>2.51±0.41</td>
<td></td>
</tr>
<tr>
<td>pHa</td>
<td>7.80±0.06</td>
<td>7.52±0.05$^\dagger$</td>
<td>7.71±0.06</td>
<td>7.73±0.06</td>
<td></td>
</tr>
<tr>
<td>$P_aO_2$ (mmHg)</td>
<td>109.8±6.5</td>
<td>135.1±5.3$^\dagger$</td>
<td>105.4±5.3</td>
<td>110.4±2.7</td>
<td></td>
</tr>
<tr>
<td>$CaO_2$ (mmol l$^{-1}$)</td>
<td>4.19±0.24</td>
<td>3.54±0.26$^\dagger$</td>
<td>4.87±0.21</td>
<td>4.88±0.27</td>
<td></td>
</tr>
<tr>
<td>[Hb] (mmol l$^{-1}$)</td>
<td>1.23±0.08</td>
<td>1.26±0.09</td>
<td>1.36±0.09</td>
<td>1.32±0.07</td>
<td></td>
</tr>
<tr>
<td>[Hb-O$_2$] (mol O$_2$ mol$^{-1}$ Hb)</td>
<td>3.25±0.06</td>
<td>2.61±0.07$^\dagger$</td>
<td>3.49±0.14</td>
<td>3.57±0.11</td>
<td></td>
</tr>
<tr>
<td>[Noradrenaline] (nmol l$^{-1}$)</td>
<td>0.36±0.15</td>
<td>ND</td>
<td>1.43±1.10</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>[Adrenaline] (nmol l$^{-1}$)</td>
<td>1.67±0.72</td>
<td>2.38±1.07</td>
<td>9.75±0.41</td>
<td>2.89±1.05</td>
<td></td>
</tr>
</tbody>
</table>

* represents a statistically significant difference from the corresponding pre-treatment value (Pre) ($P<0.05$; paired t-test); † represents a statistically significant difference from the corresponding value in the control group ($P<0.05$; unpaired t-test).

ND, not detectable.

Values are means ± S.E.M., N=6 animals.

1 mmHg=0.1333 kPa.

[Hb-O$_2$], the concentration of oxygen bound specifically to haemoglobin (Hb).
In vivo experiments

The effects of hypercapnic acidosis on catecholamine release during acute hypoxia

The effects of external hypercapnia on blood respiratory variables and plasma catecholamine concentrations are summarised in Table 2. Predictably, external hypercapnia caused a marked respiratory acidosis (decreased pH in an increase in $P_{a\text{CO}_2}$) and reductions in $C_{a\text{O}_2}$ and $O_2$ specifically bound to haemoglobin. The increase in $P_{aO_2}$ in the hypercapnic fish occurred concurrently with an apparent increase in the amplitude of opercular movements (measured by impedance changes; data not shown). Plasma catecholamine levels were unaltered by 20 min of hypercapnia.

Fig. 5 summarises the dynamics of catecholamine release during acute hypoxia under normocapnic (Fig. 5A) or hypercapnic (Fig. 5B) conditions. In each case, plasma catecholamine levels remained virtually constant at baseline levels during most of the period of exposure to graded hypoxia. However, upon reaching a critical $P_{aO_2}$ threshold, circulating catecholamine levels increased markedly and abruptly. The estimated critical $P_{aO_2}$ thresholds for catecholamine release varied greatly between the normocapnic and hypercapnic fish. For normocapnic fish, the onset of catecholamine release occurred at a $P_{aO_2}$ of approximately 15 mmHg (Fig. 5A), whereas in the hypercapnic fish, catecholamine release occurred at a $P_{aO_2}$ of approximately 33 mmHg (Fig. 5B). Hypercapnia had a pronounced influence on haemoglobin $O_2$-binding affinity as calculated from the in vivo $O_2$ equilibrium curves (Fig. 5). The $P_{50}$ values calculated for normocapnic and hypercapnic fish

![Graph](image_url)

**Fig. 4.** The effects of the muscarinic receptor agonist pilocarpine on the maximal secretion rates of (A) noradrenaline and (B) adrenaline in situ using a perfused posterior cardinal vein preparation of rainbow trout. Open columns indicate preparations continuously perfused with control saline (pH 7.9; $N=7$); filled columns indicate preparations continuously perfused with acidic saline (pH 7.4; $N=6$). ‡ denotes a statistically significant difference ($P<0.05$) from the pre-injection (Pre) value. Values are represented as means ±1 S.E.M.

![Graph](image_url)

**Fig. 5.** Plasma total catecholamine (adrenaline plus noradrenaline) levels (filled circles) during acute hypoxia in vivo under (A) normocapnic or (B) hypercapnic conditions. In each panel, the catecholamine data are superimposed upon in vivo $O_2$ equilibrium curves (open circles). Sigmoid curves were fitted to the data, and $P_{50}$ values of 11.3 and 28.3 mmHg were calculated for the normocapnic ($N=6$) and hypercapnic ($N=6$) fish, respectively. In each panel, the shaded square represents the mean plasma catecholamine concentration ±1 S.E.M. for all $P_{aO_2}$ values below 20 mmHg (normocapnic fish) and 37 mmHg (hypercapnic fish). * indicates a statistically significant difference in the mean plasma catecholamine level between the normocapnic and hypercapnic fish (t-test; $P<0.05$). Note the different plasma catecholamine $y$-axis scales in A and B. 1 mmHg=0.133 kPa.
Acid–base status and catecholamine secretion in trout were 11.3 and 28.3 mmHg, respectively. Thus, owing to the effects of hypercapnia on lowering the affinity of haemoglobin O2-binding, catecholamine release in both groups of fish corresponded with a decline in haemoglobin O2-saturation to 55–60%. It is noteworthy that the difference in the $P_{\text{aO}_2}$, catecholamine release thresholds for the normocapnic and hypercapnic fish (18 mmHg) was approximately equal to the difference in the $P_{50}$ values (17 mmHg).

Hypercapnia in vivo significantly increased the levels of plasma catecholamines achieved during acute hypoxia. The mean total catecholamine concentration during normocapnic hypoxia was 117.6±19.1 nmol l$^{-1}$ for all values corresponding with $P_{\text{aO}_2}$ values below 20 mmHg. The mean catecholamine concentration during hypercapnic hypoxia was 444.1±182.6 nmol l$^{-1}$ for all values corresponding with $P_{\text{aO}_2}$ values below 37 mmHg (the value of 37 mmHg was chosen because it differs from the normocapnic value, 20 mmHg, by the difference in $P_{50}$ values, 17 mmHg, between the two groups of fish).

Representative recordings for normocapnic and hypercapnic fish are shown in Figs 6 and 7, respectively. The onset of catecholamine release for the representative normocapnic fish occurred at a $P_{\text{aO}_2}$ of approximately 14 mmHg, and the maximal plasma catecholamine level reached was 156 nmol l$^{-1}$ (Fig. 6D). Note that the abrupt increase in plasma catecholamine levels was associated with the sudden development of metabolic acidosis superimposed upon respiratory alkalosis (Fig. 6C,D). The respiratory alkalosis is caused principally by hyperventilation, whereas the metabolic acidosis is believed to reflect the adrenergic activation of red blood cell Na$^+$/H$^+$ exchange (Fievet et al. 1990). The representative hypercapnic fish exhibited abrupt catecholamine release at a $P_{\text{aO}_2}$ of approximately 40 mmHg and achieved a maximal circulating catecholamine level of 814 nmol l$^{-1}$ (Fig. 7D).

The effects of hypercapnia on nicotine-induced release of catecholamines

Intra-arterial injections of nicotine elicited significant noradrenaline (Fig. 8A) and adrenaline (Fig. 8B) release in both normocapnic and hypercapnic fish at doses of 450 nmol kg$^{-1}$ and 600 nmol kg$^{-1}$. At the highest dose of nicotine, plasma catecholamine levels increased to significantly higher levels in the hypercapnic fish than in normocapnic fish. Values for plasma adrenaline levels in the pre-treatment and saline-injected fish were below the detection limit of the HPLC (0.1 nmol l$^{-1}$).

Fig. 6. Representative continuous in vivo recordings from a single normocapnic fish illustrating the effects of acute graded hypoxia on (A) $P_{\text{wO}_2}$, (B) $P_{\text{aO}_2}$, (C) $P_{\text{aCO}_2}$ and (D) pH and total catecholamine levels (open diamonds). The shaded area represents the period of exposure to graded hypoxia. The solid vertical line represents the abrupt onset of catecholamine release; the corresponding $P_{\text{aO}_2}$ is indicated in B. 1 mmHg=0.133 kPa.

Fig. 7. Representative continuous in vivo recordings from a single hypercapnic fish illustrating the effects of acute graded hypoxia on (A) $P_{\text{wO}_2}$, (B) $P_{\text{aO}_2}$, (C) $P_{\text{aCO}_2}$ and (D) pH and total catecholamine levels (open diamonds). The shaded area represents the period of exposure to graded hypoxia that was preceded by a 20 min interval of hypercapnia. The solid vertical line represents the abrupt onset of catecholamine release; the corresponding $P_{\text{aO}_2}$ is indicated in B. 1 mmHg=0.133 kPa.
Acute stress is often associated with marked changes in the chemical composition of the blood. In particular, physical (exhaustive exercise) and environmental (hypoxia, hypercapnia) stressors, if severe enough, may cause hypoxaemia and/or acidosis. Thus, secretion of catecholamines into the circulation at such times may occur when the chromaffin cells are exposed to acidic/hypoxic extracellular fluid. Therefore, changes in blood chemistry at the moment of catecholamine release could potentially influence secretion by a direct effect on the chromaffin tissue or indirectly by modulating the neurone-mediated reflex release pathway(s).

This is the first study to assess directly the involvement of blood acid–base status in catecholamine secretion in a teleost fish. The results demonstrated that, in rainbow trout, blood acid–base status in catecholamine secretion in a teleost release pathway(s). 

Discussion

Acute stress is often associated with marked changes in the chemical composition of the blood. In particular, physical (exhaustive exercise) and environmental (hypoxia, hypercapnia) stressors, if severe enough, may cause hypoxaemia and/or acidosis. Thus, secretion of catecholamines into the circulation at such times may occur when the chromaffin cells are exposed to acidic/hypoxic extracellular fluid. Therefore, changes in blood chemistry at the moment of catecholamine release could potentially influence secretion by a direct effect on the chromaffin tissue or indirectly by modulating the neurone-mediated reflex release pathway(s).

This is the first study to assess directly the involvement of blood acid–base status in catecholamine secretion in a teleost fish. The results demonstrated that, in rainbow trout, blood acidosis (in the physiological range) does not directly influence catecholamine secretion. However, the data obtained from in situ and in vivo experiments demonstrated that acidosis does modulate the responsiveness of trout chromaffin cells to cholinergic stimuli.

Absence of any direct effects of acidosis on catecholamine secretion

In situ experiments

The goal of these experiments was to determine whether acidification of the interstitial fluid bathing the chromaffin cells could directly affect catecholamine secretion. Because the chromaffin cells in trout are predominantly localised to the walls of the posterior cardinal vein, this was accomplished by perfusing the posterior cardinal vein with normal (pH 7.9) or acidic (pH 7.4) saline. These values were chosen because they represent typical extracellular pH values in trout at 15°C under normocapnic conditions and during periods of catecholamine release, respectively. For example, after exhaustive exercise or upon exposure to hypercapnia (i.e. $P_{CO_2}$ 7–8 mmHg), blood pH is typically reduced by 0.4–0.5 units (Wood and Perry, 1985; Wood, 1991; Perry et al. 1987). The results clearly demonstrated that catecholamine secretion was unaffected by 30 min of perfusion with acidic saline. It seems unlikely, therefore, that acidification of the blood per se during acute stress plays any direct role in the initiation of catecholamine release. However, during periods of acidosis in vivo, there are likely to be significant changes in blood metabolite (e.g. lactate) and hormone (e.g. cortisol) levels. The effects of these other variables were not assessed in the present study and thus we cannot exclude possible contributions from such metabolites and hormones to the response of chromaffin cells to acidosis.

Although similar experiments have not been performed previously on teleost fish, the results are consistent with a previous study on an agnathan, Myxine glutinosa (Perry et al. 1993). In that study, lowering the pH of the perfusate from 8.1 to 7.1 in a perfused heart preparation did not alter rates of catecholamine secretion from the cardiac chromaffin tissue. In contrast, Dashow and Epple (1985) suggested that CO2 could act as a humoral trigger of catecholamine secretion in the lamprey Petromyzon marinus. In that study, however, living fish were exposed to pure (100%) CO2 prior to withdrawing blood for analysis of catecholamines. Thus, the conclusion of a direct effect of CO2/acidosis on chromaffin tissue in the lamprey may be premature.

Studies on the effects of alterations in the acid–base status on mammalian chromaffin tissue have produced conflicting results. For example, low pH was reported to stimulate catecholamine release in the perfused adrenal medulla of the rat (Fujisawa et al. 1994). In cultured bovine adrenal chromaffin cells, however, both stimulatory (Kao et al. 1991) and inhibitory (Kruger et al. 1995) effects of acidosis have been documented.

In vivo experiments

In the present study, rainbow trout were exposed to CO2 for a short period (20 min). In one experimental series, the goal was to increase $P_{CO_2}$ to approximately 6 mmHg (final pH$a$=7.5; Table 2), whereas in another series, the goal was to
achieve a constant level of moderate external hypercapnia (P\textsubscript{WCO\textsubscript{2}}=5 mmHg). These criteria were selected on the basis of pilot experiments (A. Julio, unpublished data) showing the absence of catecholamine release at these levels of hypercapnia. Indeed, the results clearly demonstrated that plasma catecholamine levels were unaltered during hypercapnia despite the pronounced respiratory acidosis that was elicited. Several previous studies have examined the effects of hypercapnia on the circulating levels of catecholamines in trout (Perry et al. 1987, 1989; Kinkead and Perry, 1991; Kinkead et al. 1993; Thomas et al. 1994; Perry and Gilmour, 1996). The results of these studies are highly variable, with responses ranging from no elevation of catecholamine concentrations (Kinkead and Perry, 1991) to minor increases (Perry et al. 1987, 1989) to large changes (Kinkead et al. 1993; Thomas et al. 1994; Perry and Gilmour, 1996). Owing to the Root effect, a reduction in blood O\textsubscript{2} content (or haemoglobin O\textsubscript{2}-saturation) is believed to be the trigger for catecholamine release during hypercapnia in trout (Perry et al. 1989). Thus, it is possible that the differences among the studies reflect varying degrees of CO\textsubscript{2}-induced hypoxaemia. Although blood O\textsubscript{2} content was not reported in every instance, it is noteworthy that Kinkead and Perry (1991) reported no increase in plasma catecholamine levels and also detected no decrease in arterial O\textsubscript{2} concentration (C\textsubscript{aO\textsubscript{2}}). In contrast, the large increase in plasma catecholamine levels (>325 nmol l\textsuperscript{-1}) observed by Kinkead et al. (1993) was associated with a 33 % reduction in C\textsubscript{aO\textsubscript{2}}. In the present study, C\textsubscript{aO\textsubscript{2}} was reduced by 15 % after 20 min of hypercapnia; under the present conditions, this degree of hypoxaemia was presumably not severe enough to elicit catecholamine release. Other possible sources of variation between the studies may include the rate at which P\textsubscript{WCO\textsubscript{2}} is brought to equilibrium and the timing of the blood sampling.

Regardless of the differences among the studies that have examined plasma catecholamine levels in hypercapnic trout, the results of the present study strongly reinforce the notion that blood acidosis, in itself, is not a direct trigger for catecholamine secretion. Thus, in the absence of other accompanying stimuli, such as a threshold level of hypoxaemia (Perry et al. 1989), acidosis does not appear to evoke an acute adrenergic stress response in rainbow trout. Although previous studies (Boutilier et al. 1986; Tang and Boutilier, 1988) have demonstrated significant relationships between blood acidosis and plasma catecholamine levels in trout, it is not possible to differentiate between the effects of acidosis per se and the associated hypoxaemia that is induced by acidosis as a result of the Root effect.

The modulating effects of acidosis on catecholamine secretion

In situ experiments

The pH of the perfusion medium did not influence the secretory response of the chromaffin cells to carbachol, a non-specific or dual nicotinic/muscarinic receptor agonist. However, the response of the chromaffin tissue to a selective nicotinic receptor agonist, nicotine, was enhanced markedly by perfusate acidosis. These results suggested the possibility that acidosis was inhibiting the potential contribution of the muscarinic receptor to catecholamine secretion. Thus, during perfusion with acidic saline, the stimulatory effect on the nicotinic receptor was possibly being counteracted by an inhibitory effect on the muscarinic receptor. The possible involvement of the muscarinic receptor in the control of catecholamine secretion has been investigated in carp Cyprinus carpio (Gfell et al. 1997) and eel Anguilla rostrata (Reid and Perry, 1995; Al-Kharrat et al. 1997) but, prior to the present study, the contribution of the muscarinic receptor to catecholamine secretion from trout chromaffin tissue had not been assessed directly. On the basis of results showing stimulation of catecholamine secretion in the presence of the selective muscarinic receptor agonist pilocarpine, it is clear that a muscarinic receptor is present on trout chromaffin cells. However, the stimulatory effects of pilocarpine on catecholamine secretion were not reduced by acidic saline. Thus, an alternative hypothesis must be sought to explain the lack of any effect of acidosis on carbachol-evoked catecholamine secretion. The current model of catecholamine secretion in fish (for a review, see Reid et al. 1998) advocates that a number of non-cholinergic neurotransmitters and neuromodulators (e.g. vasoactive intestinal peptide, pituitary adenylate cyclase activating polypeptide) are co-released with acetylcholine during neural stimulation of the chromaffin cells. In addition, the chromaffin cells themselves release substances that are capable of eliciting autocrine and paracrine effects (serotonin, adenosine, codeine) (Epplle et al. 1994; Bernier and Perry, 1996). Therefore, it is possible that acidosis affects one or more of these non-cholinergic control systems to counter the stimulatory effect on the nicotinic receptor.

The results of the present study suggest a role for the muscarinic receptor in the cholinergic control of catecholamine secretion in trout. To demonstrate conclusively that the muscarinic receptor contributes to catecholamine secretion during neuronal stimulation of the chromaffin tissue, it would be necessary to compare catecholamine secretion during electrical stimulation of the nerves innervating the chromaffin cells in the presence and absence of a selective muscarinic receptor antagonist (e.g. atropine). The preliminary results of such experiments (C. Montpetit and S. F. Perry, unpublished observations) reveal an important stimulatory role of the muscarinic receptor. The stimulatory influence of the muscarinic receptor in trout differs from its function in the American eel Anguilla rostrata, where it is thought to have no role (Reid and Perry, 1995) or an inhibitory role (Al-Kharrat et al. 1997) in catecholamine secretion.

In vivo experiments

Intra-arterial injection of nicotine was used as a tool to evoke catecholamine release in vivo under normocapnic or hypercapnic conditions. Under hypercapnic conditions, when the blood was acidic, the highest dose of nicotine (600 nmol kg\textsuperscript{-1}) caused a significantly greater release of adrenaline and noradrenaline. These results are consistent with
the data obtained from the in situ experiments (see above) and further support the notion that catecholamine secretion, evoked by nicotinic receptor stimulation, is enhanced under acidic conditions. Because detailed dose–response curves for nicotine were not constructed either in situ or in vivo, it is not possible to determine whether acidosis affects the affinity of nicotine-evoked release. However, the increased magnitude of release at the higher doses of nicotine suggests that acidosis up-regulates the nicotinic receptor or one or more downstream components in the signal-transduction pathway leading to catecholamine secretion.

Prior exposure of hypoxic fish to hypercapnia caused a pronounced decrease in the affinity of haemoglobin O₂-binding. Current theory contends that catecholamine release during exposure of trout to acute hypoxia occurs abruptly as \( P_{O_2} \) falls below a critical threshold corresponding to a reduction in haemoglobin O₂-saturation of 40–50% (Perry and Reid, 1992, 1994; Thomas and Perry, 1992; Thomas et al. 1992). Thus, in the absence of any direct effects of acidosis on catecholamine release, it was predicted that hypercapnia would increase the \( P_{O_2} \) threshold for secretion by an amount equivalent to the increase in the \( P_{50} \) value; this is what was observed. Regardless of the acid–base status of the blood, the threshold for catecholamine release occurred at approximately 55–60% haemoglobin O₂-saturation. Thus, we conclude that acidosis, in itself, does not alter the affinity of nicotine-evoked release. However, the increased magnitude of release to determine whether acidosis affects the affinity of nicotine-receptor or one or more downstream components in the signal-transduction pathway leading to catecholamine secretion.

This work was financed by Natural Sciences and Engineering Research Council (NSERC) of Canada Research and Equipment grants to S. F. Perry. A. Julio and C. Montpetit would like to thank N. Bernier, M. Furimsky, J. McKendry and P. D. Spencer for all their help and encouragement throughout this project.

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