CONTRIBUTION OF THE SECONDARY CIRCULATORY SYSTEM TO ACID–BASE REGULATION DURING HYPERCAPNIA IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

BY ATSUSHI ISHIMATSU*, GEORGE K. IWAMA†, TIMOTHY B. BENTLEY‡ AND NORBERT HEISLER

Abteilung Physiologie, Max-Planck-Institut für experimentelle Medizin, Hermann-Rein-Straße 3, D-3400 Göttingen, FRG

Accepted 16 April 1992

Summary

The contribution of the secondary circulatory system to acid–base regulation and epithelial ion transport was evaluated qualitatively in freshwater-acclimated rainbow trout. The dorsal aorta (DA) and the lateral cutaneous vessel (LCV) (which is considered to be the venous drainage of the secondary system) were chronically cannulated and the fish were exposed to environmental hypercapnia (2% CO₂) after establishment of normocapnic control values. Fluid sampled from the LCV contained much less haemoglobin (0.14 g 100 ml⁻¹) and fewer blood cells (packed cell volume, PCV, 1.2–1.7%) than DA blood ([Hb] 8.2–8.9 g 100 ml⁻¹, PCV 27.2–32.5%) regardless of ambient CO₂ levels, indicating highly limited access of red blood cells to the secondary circulatory system through anastomoses connecting it to the primary system. There was no significant difference between the two sampling sites for any of the acid–base variables (pH, P CO₂, [HCO₃⁻]) and most plasma ion concentrations ([Na⁺], [Ca²⁺], [Mg²⁺] and [Cl⁻]) during normocapnia, although plasma [K⁺] and osmolarity were significantly lower in LCV fluid. Upon exposure to hypercapnia, the pH of LCV fluid became significantly higher than that of DA plasma because of the higher [HCO₃⁻] in the LCV plasma than in the DA plasma. The LCV plasma concentration of Cl⁻ was significantly lower than that of DA plasma during hypercapnia. These data suggest that the secondary circulatory system draining the body surface is involved in ionic acid–base regulation in fish, probably via Cl⁻/HCO₃⁻ exchange processes across the extrabranchial epithelium of the body surface.

* Present address: Nomo Fisheries Station, Nagasaki University, Nomozaki, Nagasaki 851-05, Japan.
† Present address: Department of Animal Science, University of British Columbia, 248-2357 Main Mall, Vancouver, BC, Canada V6T 2A2.
‡ Present address: Department of Physiology, Medical College of Virginia, Box 551, MCV, Richmond, VA 23928, USA.

Key words: secondary circulatory system, acid–base regulation, ionic transfer, body surface epithelium, rainbow trout, Onchorhynchus mykiss.
Introduction

Recent anatomical studies have cast doubts on the nature of the so-called lymphatic system in fish. Close inspection of vascular casts of five species of fish (Vogel, 1981; Vogel and Claviez, 1981) as well as in vivo observation of the microcirculatory system in the transparent glass catfish (Steffensen et al. 1986) revealed the so-called lymphatic vessels to be the drainage conduits of a unique vascular system, designated by Vogel (1981) as the ‘secondary circulatory system’. Flow into the secondary system originates from arteries of the primary circulatory system via numerous capillary-sized (inner diameter 10–15 μm) anastomoses (Steffensen et al. 1986). The secondary system itself consists of distributing, exchange and collecting vessels analogous to the arteries, capillaries and veins of the primary system, respectively. Capillaries of the secondary system appear to be distributed mainly in the internal and external body surfaces, such as the mucosa of the skin and pharynx, as well as the peritoneum. Vogel (1985) demonstrated that dense networks of secondary capillaries exist above the scales, directly underlying the skin epithelium of trout and tilapia. Sixteen years earlier, an intricate capillary network beneath the epidermis of the exposed part of each scale was described for pike by Tysekiewicz (1969); a system probably analogous to the superficial secondary capillaries described by Vogel (cf. Satchell, 1991).

Based on the distribution of secondary capillaries, Vogel (1985) and Satchell (1991) have suggested that the secondary system may play a role in transepithelial ion transport. The strikingly close juxtaposition between the large surface area of skin secondary capillaries with the epithelium and the external environment implies strongly that the body surface area is a potentially important site for ion and acid–base regulation. The lack of any relevant physiological studies on this subject may be related to the methodological difficulties in isolating the fluid from the secondary system. The aim of the present study was to compare acid–base status and ion concentrations of the secondary vascular system fluid with those of blood plasma from the primary circulatory system at rest and during acid–base disturbance. For this purpose, the dorsal aorta (DA) and the lateral cutaneous vessel of the secondary vascular system (LCV, ‘lateral lymphatic’ according to Kampmeier, 1969) were chronically cannulated in rainbow trout (Oncorhynchus mykiss). Animals were, after a normocapnic control period, subjected to environmental hypercapnia in order to elucidate the contribution of the secondary vascular system to the regulation of acid–base homeostasis during this state of stimulated transepithelial ion transport.

Materials and methods

Experimental animals

Rainbow trout [Oncorhynchus mykiss (Walbaum)] were obtained from a hatchery near Köln (Germany), and kept for several weeks to several months in large, well-aerated glass aquaria (2–3 m³) at 15°C with a continuous supply of dechlorinated and aerated Göttingen tap water (100–200 ml fish⁻¹ min⁻¹; ionic
Secondary circulatory system and ionic regulation

composition: \([\text{Na}^+] 0.35-0.4 \text{mmol} \text{l}^{-1}, \ [\text{Cl}^-] 0.3-0.35 \text{mmol} \text{l}^{-1}, \ [\text{HCO}_3^-] 0.8-1.2 \text{mmol} \text{l}^{-1}, \ [\text{Ca}^{2+}] 0.7-1 \text{mmol} \text{l}^{-1}, \ \text{pH} 7.7-8.1\). Body mass ranged from 675 to 1150 g. The fish were maintained on a commercial trout diet until 2 days prior to surgery.

**Surgery**

The fish were anaesthetized by immersion in a solution of 0.01\% MS-222 in Göttingen tap water, buffered by addition of NaHCO\(_3\). During surgery, the gills were irrigated to maintain anaesthesia with a solution of 0.005\% MS-222 solution, bubbled with pure \(\text{O}_2\) and thermostatted to 15\(^\circ\)C.

The dorsal aorta (DA) was cannulated with PE50 catheters by application of a modified Seldinger technique, similar to the procedure of Soivio *et al.* (1975). Following DA cannulation, the LCV was non-occlusively cannulated. The vessel was exposed by a short (7-10 mm) dorso-ventral incision in the skin, slightly dorsal to the lateral line at the level of the rostral margin of the dorsal fin. By careful dissection and retraction, the vessel was exposed with very little damage to the subcutaneous tissue. T-pieces, constructed from PE50, were inserted into the LCV. Both tips of the horizontal bar of the T-piece were covered with silastic tubing of the same diameter to facilitate insertion. The wound was closed with fineatraumatic silk sutures (6/0). The cannulae were filled with heparinized (50 i.u. ml\(^{-1}\)) Cortland saline (Wolf, 1963) without glucose or with similarly heparinized 0.9\% NaCl solution. Surgery took about 20 min.

After surgery, the fish were allowed to recover for at least 20 h in a recirculating water system consisting of a fish chamber and a 200 l reservoir. The water in the reservoir was vigorously aerated, thermostatted to 15\(^\circ\)C, and circulated through the chamber at a flow rate of 51 min\(^{-1}\). The fish chamber was darkened to prevent visual disturbance to the fish.

**Protocol**

After two control samples had been taken, 3 h apart, the system was equilibrated with 2\% \(\text{CO}_2\) (water \(P_{\text{CO}_2}\) stabilized at the new level within 5–7 min due to counter-current water/gas flow in our system, vigorous aeration of 7–121 min\(^{-1}\) and the high recirculation rate), and subsequent samples were taken 1 and 4 h after the onset of hypercapnia. For DA sampling, the first 1 ml was withdrawn with a syringe to clear the cannula of saline and the following 800 \(\mu\)l was collected in an Eppendorf vial by siphoning. The vial was centrifuged at 16 000 \(g\) to obtain plasma for ion analyses. LCV fluid was collected by siphoning, discarding the first 1 ml to clear the cannula and collecting the next 800 \(\mu\)l, which was treated in the same way as the DA samples. Two microhaematocrit tubes were filled from each cannula for acid–base measurements.

**Analytical methods**

pH was determined using a glass microcapillary electrode thermostatted to the animal’s body temperature and standardized with precision phosphate buffers
(Radiometer BMS3 Mk2). Samples of blood and LCV fluid were centrifuged anaerobically at 16,000 g and total CO₂ ($T_{CO_2,pl}$) of the supernatant was determined by measurement of differential conductivity, utilizing a Capnicon III instrument (Cameron Instruments, Port Aransas, TX, USA). The partial pressure of CO₂ ($P_{CO_2}$) in the plasma was calculated from measured pH and $T_{CO_2}$ values applying the Henderson–Hasselbalch equation with appropriate constants (Heisler, 1984, 1990) (note: the last line term of the formula for CO₂ solubility in Heisler, 1984, is misprinted and should read ‘+’). Plasma $[HCO_3^-]$ was calculated by subtracting the fraction of molecular CO₂ from $T_{CO_2}$. Haemoglobin concentration ([Hb]) was determined by the cyanmethaemoglobin method (Sigma Diagnostics kit no. 525). Packed cell volume (PCV: haematocrit plus white cells) was measured after centrifugation at 16,000 g for 5 min. Osmolarity was determined with a Wescor 5100C vapour pressure osmometer. Plasma concentrations of sodium ([Na\(^+\)\(_{pl}\)], potassium ([K\(^+\)\(_{pl}\)], magnesium ([Mg\(^{2+}\)\(_{pl}\)]), and calcium ([Ca\(^{2+}\)\(_{pl}\)] were measured by atomic absorption spectrophotometry (Perkin-Elmer model 2380), and plasma chloride concentration ([Cl\(^-\)\(_{pl}\)] was determined by coulometric titration (digital chloride titrator; Haake Buchler Instruments, New Jersey, USA). Plasma protein concentration ([protein]\(_{pl}\)] was analyzed colorimetrically (Sigma Diagnostics kit no. 540). DA and LCV pressures were continuously recorded between samples using blood pressure transducers (Statham P23PB) and a chart recorder (Gould 2600S).

**Statistical analysis**

In order to circumvent the large interindividual variability a paired t-test was used for comparison between data taken simultaneously from the two sampling sites (DA and LCV) (cf. Zar, 1984). Statistical differences between values obtained during control and hypercapnic periods were assessed for each site by the method of Dunnett (1964), the method of choice suggested by Wallenstein et al. (1980). Data are generally expressed as mean±s.e.

**Results**

**Control condition**

Control values of 12 measured variables in DA blood and LCV fluid are summarized in Table 1. Although all of the measured variables tended to differ between the two sites, only PCV, [Hb] (Fig. 1), [K\(^+\)]\(_{pl}\) (Fig. 3), and osmolarity were significantly different. Since LCV fluid contained very few red blood cells, a large fraction of the packed cell volume presented in Table 1 is attributable to white blood cells. $P_{CO_2}$ and $[HCO_3^-]_{pl}$ of the fish used in the present study were high for rainbow trout. Data were confirmed repeatedly by different operators using a number of instruments. Since similar data were also obtained from some fish with only DA cannulae, the LCV cannulation apparently did not affect the acid–base and ionic status of the animal to any significant extent. Thus, the
Table 1. Comparison of measured variables between the dorsal aortic blood plasma (DA) and lateral vessel fluid (LCV) of freshwater-acclimated rainbow trout under control conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>DA</th>
<th>LCV</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid–base status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>(kPa)</td>
<td>8.080±0.064</td>
<td>7.942±0.089</td>
<td></td>
</tr>
<tr>
<td>$P_{CO_2}$</td>
<td>(mmHg)</td>
<td>0.55±0.03</td>
<td>0.87±0.11</td>
<td></td>
</tr>
<tr>
<td>$[HCO_3^-]_{pl}$</td>
<td>(mmol/l)</td>
<td>4.1±0.22</td>
<td>6.5±0.86</td>
<td></td>
</tr>
<tr>
<td>Haematology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td></td>
<td>27.2±1.92</td>
<td>1.7±0.33</td>
<td>$P&lt;0.01$</td>
</tr>
<tr>
<td>$[Hb]$</td>
<td>(g/100 ml$^{-1}$)</td>
<td>8.2±0.58</td>
<td>0.14±0.02</td>
<td>$P&lt;0.01$</td>
</tr>
<tr>
<td>$[Protein]_{pl}$</td>
<td>(g/100 ml$^{-1}$)</td>
<td>5.9±0.87</td>
<td>3.7±0.39 (5)</td>
<td></td>
</tr>
<tr>
<td>Ionic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[Na^+]_{pl}$</td>
<td>(mmol/l$^{-1}$)</td>
<td>144.8±3.43</td>
<td>143.9±4.0</td>
<td></td>
</tr>
<tr>
<td>$[K^+]_{pl}$</td>
<td>(mmol/l$^{-1}$)</td>
<td>3.5±0.16</td>
<td>2.4±0.18</td>
<td>$P&lt;0.01$</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{pl}$</td>
<td>(mmol/l$^{-1}$)</td>
<td>2.3±0.17</td>
<td>2.2±0.12</td>
<td></td>
</tr>
<tr>
<td>$[Mg^{2+}]_{pl}$</td>
<td>(mmol/l$^{-1}$)</td>
<td>0.81±0.03</td>
<td>0.80±0.02 (5)</td>
<td></td>
</tr>
<tr>
<td>$[Cl^-]_{pl}$</td>
<td>(mmol/l$^{-1}$)</td>
<td>117.1±4.86</td>
<td>115.3±4.5</td>
<td></td>
</tr>
<tr>
<td>Plasma osmolarity</td>
<td>(mosmol/l$^{-1}$)</td>
<td>284.2±2.73</td>
<td>277.9±0.6</td>
<td>$P&lt;0.05$</td>
</tr>
</tbody>
</table>

Values are mean±s.e., $N=6$ unless given in parentheses.
Significant difference between the LCV value and the corresponding DA value are calculated by paired t-test.

somewhat unusually high $[HCO_3^-]$ and $P_{CO_2}$ values have to be attributed to strain variability or to a hypercapnic state of unknown cause.

Response to hypercapnia

As a characteristic response, the PCV of DA blood increased significantly after exposure to hypercapnia, whereas the simultaneous rise in $[Hb]$ and the fall in $[protein]$ were statistically insignificant (Fig. 1). Hypercapnia did not affect the significance of differences between DA and LCV values for PCV and $[Hb]$. During hypercapnia, pH and $[HCO_3^-]_{pl}$ of LCV fluid became significantly higher than the corresponding values for DA blood, although the $[HCO_3^-]_{pl}$ difference was statistically significant only after 4h of hypercapnia (Fig. 2). There were no differences in $P_{CO_2}$ between sample sites. After the onset of hypercapnia, the LCV $[Cl^-]_{pl}$ became significantly lower than the DA plasma concentration, whereas $[Na^+]_{pl}$ and $[K^+]_{pl}$ remained essentially unaffected (Fig. 3). $[Mg^{2+}]_{pl}$ was slightly, but significantly, increased at both sites (approximately 0.1 mmol/l$^{-1}$) after 4h, but there was no significant difference between sites. A significant difference in $[K^+]_{pl}$ between sites under control conditions vanished upon exposure to hypercapnia, and the DA concentration was significantly decreased at 1h of hypercapnia. $[Ca^{2+}]_{pl}$ was unaffected by hypercapnia, indicating, together with the data on $[Mg^{2+}]$, that osteal structures were not involved in the accumulation of $HCO_3^-$.
during hypercapnia. Osmolarity in the DA fell slightly during hypercapnia, eliminating a small but significant difference from that of the LCV found under control conditions.
Secondary circulatory system and ionic regulation

Hypercapnia did not significantly affect cardiovascular variables. Heart rate \((f_h)\) and average DA blood pressure \((P_{DA})\) were the same under control and hypercapnic periods \([f_h 64.7\pm6.5\text{ and } 61.6\pm6.5\text{ min}^{-1}; P_{DA} 41.2\pm1.7\text{ and } 41.4\pm2.0\text{ cmH}_2\text{O (0.404\pm0.017 and 0.406\pm0.20 kPa)}, N=5, \text{ respectively}]\). LCV pressure was very low \((-1.3 \text{ to } 3.8\text{ cmH}_2\text{O; } -0.2 \text{ to } 0.5\text{ kPa})\) and somewhat variable irrespective of CO\(_2\) levels.

Discussion

The important role of transepithelial ion transfer for acid–base regulation in fish is well established. Electroneutral \(\text{Cl}^-/\text{HCO}_3^-\) and \(\text{Na}^+/\text{NH}_4^+ (\text{H}^+)\) exchange mechanisms are generally accepted as the mechanisms predominantly responsible for compensation of exogenous and endogenous acid–base perturbations (Evans, 1986; Heisler, 1986a), but an electrogenic transepithelial \(\text{H}^+\) pump has recently been postulated to be involved as well (Avella and Bornancin, 1989; Lin and Randall, 1991). Although a general understanding of the ion movements during acid–base regulation in fish has been established during the past ten years, our knowledge of the underlying physiological mechanisms is incomplete, and the amount of information concerning sites of transepithelial transfers remains scant. A number of studies have been conducted to evaluate the role of the kidneys in fish acid–base regulation and these have generally indicated a minor contribution (for a review, see Heisler, 1986a). The gills, with their large epithelial surface area, short blood–water distances and high rates of perfusion and ventilation, are accordingly thought to be primarily responsible for the observed ion transfers. A possible contribution of cutaneous exchange sites has been assessed to be minor (<1%) in at least one species of elasmobranch fish (Heisler et al. 1976), but analogous experiments have never been performed in teleosts.

Fish skin is generally considered to be poorly permeable to ions and water (Fromm, 1968), but a few studies have indicated a significant role of the skin for transfer of ions not related to acid–base regulation. In resting freshwater rainbow trout about 50% of the Ca\(^{2+}\) uptake takes place over the general body surface (Perry and Wood, 1985), and the Cl\(^-\) efflux through the skin of the seawater shanny (Blennius pholis) accounted for 65% of the total efflux (Nonnotte et al. 1979). An earlier study by Mashiko and Jozuka (1964) demonstrated that the fins were involved in Ca\(^{2+}\) uptake and release, but more so in a seawater species (Duymaeria flagellifera) than in a freshwater species (Carassius carassius).

Chloride cells are considered to be responsible for most of the transepithelial
Fig. 2. Plasma pH, \(P_{CO_2}\) and \([HCO_3^-]\) in dorsal aortic blood (DA) and lateral cutaneous vessel fluid (LCV) of freshwater rainbow trout during environmental hypercapnia (2% CO\(_2\)). Mean±s.e., \(N=6\). Asterisks denote significantly different values from the corresponding controls at the same site (\(** P<0.01\), Dunnett's test). Daggers denote a significant difference between the two sites at corresponding times (\(\dagger P<0.05\), \(\ddagger P<0.01\), paired \(t\)-test).
ion transfer and they occur in the skin of several fish species (Hwang, 1989; Henrikson and Matoltsy, 1968; Stiffler et al. 1986; Nonnotte et al. 1979), suggesting that fish skin may be involved in the ion exchange processes required for acid–base homeostasis. In special cases, chloride cells occur in large numbers in specialized body surface areas. The opercular epithelium of Fundulus heteroclitus (Karnaky and Kinter, 1977) and Sarotherodon mossambicus (Foskett et al. 1981) and the skin of Gillichthys mirabilis (Marshall, 1977) are such sites, which have been shown by...
in vitro studies to be the location of high ion transfer activity. However, the serosal environment of those rich chloride cell populations must be in very close proximity to the vasculature of the secondary circulatory system to allow for effective exchange between the interstitial fluid and the bulk extracellular fluid. Such an arrangement is seen in the gills, where the central venous sinus directly underlies the filamental epithelium containing the chloride cells.

Although the juxtaposition of chloride cells and secondary circulation has not yet been demonstrated, the abundance of ion-transporting epithelial cells on the body surface would be a rather ineffective system of ion transfer without the support of an underlying functional convective system. A relatively high rate of convective mixing will be a critical factor for an effective link between ion-transferring epithelia and the bulk extracellular fluid. This is particularly important with respect to the establishment of ionic gradients. A low rate of convective mixing will result in a build-up of ions in the interstitial fluid, reducing the ion transfer velocity by increased electrochemical gradients, or it may even effect complete shut-down of any transfer until the accumulated ions are removed by convective mechanisms, similar to those observed during lactacidosis in skeletal muscle tissue (perfusion/equilibrium limitation; Heisler, 1986b). Perfusion of the secondary vascular system with 'skimmed' blood containing very few red cells may provide a much more effective transport vehicle, because of the lower viscosity of LCV fluid. This will result in higher flow rates at lower perfusion pressures, providing a considerable saving of cardiac energy (Satchell, 1991). As observed by Vogel (1985), and shown in this study, the fluid from the secondary system has a low haematocrit, supporting this view of perfusion energetics. Similar differences, although not as pronounced, have been reported between the ventral aorta and the subcutaneous sinus of hagfish, and this has been postulated to be the evolutionary forerunner of the cutaneous vessels in teleosts (Forster et al. 1989).

However, transport of ions is energetically expensive, and tissues involved in ionic regulation are generally characterized by a substantial metabolic rate. Since fluid pumped through the secondary system will, owing to the lack of red blood cells, not provide any reasonable transport medium for oxygen, oxygen has to be taken up directly from the ambient water. Fish skin consumes a considerable amount of oxygen directly from the environment (Kirsch and Nonotte, 1977; Nonotte and Kirsch, 1978; for a review, see Feder and Burggren, 1985), indicating a high metabolic activity probably related to ion transfer processes.

The secondary circulatory system originates from the arterial side of the primary system and, therefore, the ionic composition of the secondary arterial plasma should be identical to that of the arterial plasma in the primary circulation, as long as ionic exchange with the environment does not take place. In this study, we have collected samples from the lateral cutaneous vessel, which is regarded as the venous drainage of the secondary system. Although we have not examined the direction of fluid flow in the LCV, its open connection with the common cardinal vein at the rostral end of the LCV (Kampmeier, 1969; Wardle, 1971) and the near absence of red blood cells in LCV fluid (Table 1, Fig. 1) are compatible only with
Secondary circulatory system and ionic regulation

forward flow in the vessel, at least in its anterior portion. Accordingly, any difference in acid-base and ionic status from the fluid of the primary system (DA plasma) should reflect ion exchange processes through the capillary walls of the secondary circulatory system. We postulate that the LCV would receive fluid collected exclusively from the segmental veins of the secondary system after it has traversed the capillary networks in the skin. Under these conditions, differences in ionic and acid-base composition have to be ascribed to transcutaneous transport. A possible small admixture of fluid from deep secondary vessels, however, cannot be ruled out.

During normocapnia, acid-base status and electrolyte concentrations were almost identical at the two sites, except for \([K^+]_{pl}\) and osmolarity (Table 1). The basis for these differences is unclear and the mean difference in osmolarity (6 mosmol l\(^{-1}\)) cannot be explained by the difference in \([K^+]_{pl}\) alone. Thus, under control conditions the skin of trout does not appear to be significantly involved in ion transfer as far as net ion fluxes are concerned. In contrast, hypercapnia induced a significant difference in pH between the two sites (LCV pH > DA pH) in spite of identical \(P_{CO_2}\) values (Fig. 2). This was brought about by a higher LCV \([HCO_3^-]_{pl}\). Based on the average difference of 2.1 mmol l\(^{-1}\) between the DA and LCV, and the amount of \(HCO_3^-\) produced by non-bicarbonate buffering due to the shift in pH (0.1 mmol l\(^{-1}\), buffer line according to Wood et al. 1982), about 2.2 mmol l\(^{-1}\) of bicarbonate has been gained after 4 h of hypercapnia. This concentration difference corresponds very well with the reduction in \([Cl^-]_{pl}\) (2.4 mmol l\(^{-1}\)) (Fig. 3). This suggests a net \(Cl^-/HCO_3^-\) exchange in the secondary circulatory system of the body surface. A similar relationship between positive \([HCO_3^-]\) differences and negative \([Cl^-]\) differences has repeatedly been observed in plasma of fish during exposure to hypercapnia (Toews et al. 1983; Perry et al. 1987; Cameron and Iwama, 1987; Iwama and Heisler, 1991; G. K. Iwama, A. Ishimatsu and N. Heisler, in preparation), whereas the effect of hypercapnia on other plasma ion concentrations is less consistent.

Although the above data strongly suggest the involvement of the secondary circulatory system in the acid-base regulation during hypercapnia, its quantitative role in the overall process may not be large. Recently, Steffensen and Lomholt (1992) estimated the perfusion rate in the secondary system based on data from the distribution of labelled plasma proteins between the primary and secondary systems to be 6.6 ml kg\(^{-1}\) h\(^{-1}\). This is equivalent to 0.3% of cardiac output. This may indicate a rather limited physiological significance of the epithelial ionic transport mediated by the secondary circulatory system. On the basis of this estimate of perfusion rate and the bicarbonate concentration difference reported in this study, the \(HCO_3^-\) gain through the secondary system would amount to only 0.05 mmol kg\(^{-1}\) for 4 h of hypercapnia, which represents, in relation to the total extracellular gain during 4 h of 1.26 mmol kg\(^{-1}\) (\(\Delta[HCO_3^-]_{DA} \times \text{relative extracellular volume} = 6.3 \times 0.2 \text{mmol kg}^{-1}\)), a contribution of only 4%. However, it must be kept in mind that the above perfusion rate has been obtained from resting rather than hypercapnia-stressed fish. Recent data from our experiments indicate
that the gill filamental epithelium overlying the central venous sinus accounts for a
major fraction of the bicarbonate gained from the environment during hypercap-
nia in rainbow trout (G. K. Iwama, A. Ishimatsu and N. Heisler, in preparation);
such data certainly diminish the possibility of a large contribution from the
secondary circulation system at the body surface. However, the estimated relative
contribution is not in accordance with the high rate of skin oxygen consumption,
which can only be attributed to ion transport. Also, the magnitude of diffusional
ion exchange between the secondary and primary (venous) systems is still
unknown. Further studies are required for a quantitative evaluation of the role of
this subsystem in ionic and acid–base regulation.

Determination of LCV flow using labelled proteins may not be an appropriate
method for measuring ‘effective LCV convection’. We injected Methylene Blue
into the secondary system of one anaesthetized trout via a cannula occlusively
inserted into the caudal part of the lateral cutaneous vessel at the level of the
pectoral fin. On dissection, it became evident that the dye was almost exclusively
located in the kidneys. This is in accordance with the report of Kampmeier (1969)
stating that the LCV of teleosts is connected to the deep ‘lymphatics’ running
alongside the kidneys at the level of the caudal extremity of the abdominal cavity
(called the ‘circumanal lymph plexus’). Flow of the dye from the lateral cutaneous
vessel through the circumanal plexus to the kidneys indicates the path of least
resistance. Unfortunately, the natural direction of flow through the plexus,
whether from the kidneys to the LCV or in the opposite direction, is unknown, as
is the connection or distribution of the secondary system within the capillary
networks of the kidneys. The vascular connection through the circumanal plexus
may represent a functional linkage between the kidneys and the secondary surface
capillary bed. The role of the secondary system may be that of a more or less
closed secondary convective pool, facilitating transfer or ions from the fluid
bathing the ion-transporting cells of the body surface to the primary system. Flow
in this system may be induced by pulsations of the primary vasculature, by local
pressure changes induced by muscular activity, and/or by the activity of lymph
hearts. Convective mixing of secondary vascular fluid between the body surface
and the kidneys could affect the composition of the extratubular interstitial fluid,
thus providing a special extracellular environment for ion-transporting kidney
cells. The close relationship between the secondary circulation and the kidney
tissues may accordingly allow for elimination of protons to (or gain of bicarbonate
from) the secondary system with modifications of renal effluent plasma that are
not reflected by changes in urine composition. With such an interrelationship
between the primary and secondary systems, the kidneys may have a much larger
role in acid–base regulation than thought previously. An evaluation of their
physiological role by urinary catheter alone may prove to be inadequate.

Supported by Deutsche Forschungsgemeinschaft. A.I., G.K.I. and T.B. were
supported by the Max-Planck-Gesellschaft, and G.K.I. received an International
Collaborative Research Grant for travel from the Natural Science and Engineer-
Secondary circulatory system and ionic regulation

ing Research Council of Canada. The skillful technical assistance of Ms Dorit Alborn and Mr G. Forcht are gratefully acknowledged.

References


