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SHORT COMMUNICATION

PRE- AND POSTBRANCHIAL CARBON DIOXIDE CONTENT OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) BLOOD AFTER CATECHOLAMINE INJECTION

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It is generally accepted that plasma bicarbonate is the major source of carbon dioxide excreted in the gills of teleost fish (Perry, 1986). Although anion exchange across the membrane of rainbow trout erythrocytes is rapid, with a half-time of 0.8s for chloride equilibration at 15°C (Romano and Passow, 1984), the rate of bicarbonate influx into the erythrocytes limits the rate of conversion of plasma bicarbonate to carbon dioxide and, thereby, carbon dioxide excretion per unit volume of blood in gills, because the residence time of blood in the secondary lamellae of the gills is only 1–6s (Hughes *et al.* 1981; Bhargava *et al.* 1992). Thus, factors that reduce the net rate of bicarbonate influx through the anion exchanger may reduce the efficiency of carbon dioxide excretion in gills. The effect is, however, temporary. If carbon dioxide production remains constant, the reduction of carbon dioxide excretion will increase the venous carbon dioxide tension and content, thus increasing the diffusion gradient across the gills and speeding up CO_2 removal until the CO_2 excretion again matches production.

An inhibition of the net conversion of plasma bicarbonate to carbon dioxide by catecholamines was observed by Wood and Perry (1991) and Perry *et al.* (1991) in careful *in vitro* experiments in which the carbon dioxide gradients resembled those of the gills. It is unclear at the present time whether catecholamines transiently reduce carbon dioxide excretion in gills *in vivo*. Steffensen *et al.* (1987) could not observe any inhibition of carbon dioxide excretion in rainbow trout after an injection of catecholamines into the bloodstream when they measured the total carbon dioxide content of the water entering and leaving the respirometer. Similarly, Playle *et al.* (1990) did not observe any inhibition of carbon dioxide excretion after catecholamine injection when they measured the carbon dioxide content of water entering and leaving the gills using opercular cannulae. In contrast, the above *in vitro* findings, together with the observations (1) that the dorsal aortic carbon dioxide tension of teleost fish increases after exercise (see, for example, Wood and Perry, 1985), although the oxygen tension is not affected, and (2) that the liberation of endogenous catecholamines or injection of exogenous catecholamines into the bloodstream of hypoxic rainbow trout caused an increase in the measured carbon dioxide tension, which could be inhibited by β-adrenergic antagonists (Perry and

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Thomas, 1991), were taken to indicate that catecholamines reduce the overall carbon dioxide excretion in gills (e.g. Perry and Wood, 1989; Perry and Thomas, 1991).

It is apparent that measurements of carbon dioxide tension in post-branchial blood cannot give conclusive evidence about carbon dioxide excretion in the gills per unit volume of blood. For example, Nikinmaa and Jensen (1986) observed an increase in the post-branchial carbon dioxide tension after exercise yet, during passage through the gills, the total carbon dioxide content of plasma decreased more after exercise than at rest, indicating an increase in the excretion of carbon dioxide per unit volume of blood. A full evaluation of carbon dioxide excretion would require continuous measurements of both total carbon dioxide content of blood entering and leaving the gills and cardiac output. However, since catecholamines do not appear to affect the overall oxygen consumption (and carbon dioxide production) significantly (Steffensen *et al.* 1987), even a transient carbon dioxide retention in gills should be seen as (1) an increase in pre- and post-branchial total carbon dioxide content and (2) a reduction in the efficiency of carbon dioxide removal at the gills, provided that the measurements are carried out at times during which the occurrence of transient phenomena could be expected. By analogy with the oxygen extraction coefficient, $[C_{O_2}(\text{inspired water}) - C_{O_2}(\text{expired water})]/C_{O_2}(\text{inspired water}) \times 100$ (e.g. Dejours, 1975), the efficiency of carbon dioxide removal in gills can be given as 'carbon dioxide clearance coefficient', i.e. $[C_{CO_2}(\text{pre-branchial blood}) - C_{CO_2}(\text{post-branchial blood})]/C_{CO_2}(\text{pre-branchial blood}) \times 100$.

In the present study, the pre- and post-branchial total carbon dioxide content of blood in resting rainbow trout was measured before and after noradrenaline had been injected into the bloodstream of the animals. Thus, it was possible to evaluate the effects of catecholamines both on the total carbon dioxide content of blood and on the carbon dioxide clearance coefficient. A very high (10^{-5} mol l^{-1}) nominal concentration of noradrenaline was used to achieve a maximal effect, since Perry *et al.* (1991), for example, have shown that the effect of noradrenaline on the conversion of plasma bicarbonate to carbon dioxide increases at least up to a concentration of 10^{-6} mol l^{-1}.

Rainbow trout (*Oncorhynchus mykiss*; *N*=26; mass 544±21g; mean ± S.E.M.) were obtained from a commercial fish farm and maintained in laboratory conditions (pH7.5; temperature 9°C; carbon dioxide tension <66.7Pa; oxygen tension >16kPa) for 2 weeks before the experiments. Catheters were placed in both the dorsal and the ventral aorta, as described by Nikinmaa and Jensen (1986), and the animals were allowed to recover from the operation for 48h in individual flow-through boxes before the experiments.

At the onset of the experiments, 0.3ml of blood was taken simultaneously from both the dorsal and the ventral aortae (within 2min), whereafter 1mmol l^{-1} noradrenaline solution (in physiological saline) was injected into the animals *via* the dorsal aortic cannula to give an approximate final nominal concentration of 10 μmol l^{-1} in the blood (based on 5% blood volume, injected volume 0.2–0.3ml). The injected dose invariably caused a colour change in the animals. Blood samples (0.3ml) from both aortae were taken 5min (range 4–6min) and 30min (range 29–31min) after the injection of noradrenaline. To ensure that the bolus injection did not cause the observed changes, another group of fish was sampled, injected with 0.2–0.3ml of physiological saline, and sampled 5 and 30min after the injection.

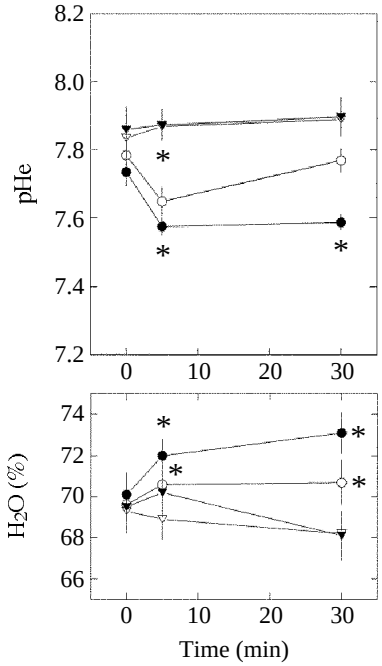

Fig. 1. The plasma pH (pHe) and red cell water content [H_2O (%)] of dorsal (open symbols) and ventral (filled symbols) aortic blood in saline-injected (triangles) and noradrenaline-treated (final nominal concentration approximately 10^{-5} mol l^{-1}; circles) rainbow trout before (0min) and 5 and 30min after the injection. Mean ± S.E.M. are given. For saline-injected fish, N=6; for noradrenaline-injected animals, N=16. Asterisks indicate the statistical significance (P<0.05) of the difference between the mean obtained before injection of noradrenaline and 5 or 30min after the injection. A Wilcoxon matched-pairs signed-ranks test was used for comparisons, since the values were not normally distributed.

Immediately after sampling, the total carbon dioxide content of blood was determined by Cameron's (1971) method, using two Radiometer carbon dioxide electrodes and PHM 72 and PHM 73 analyzers. Plasma pH was measured using a Radiometer capillary electrode. Plasma and red cells were separated by centrifugation (11000***g***, 2min) and the water content of the cell pellet was determined by weighing, drying to a constant weight (48h at 80°C) and reweighing (Nikinmaa and Huestis, 1984). The values obtained were not corrected for extracellular trapped water. From the carbon dioxide content data, the carbon dioxide clearance coefficient was calculated for every fish at every time point using the formula: [C_{CO_2tot}(pre-branchial blood) − C_{CO_2tot}(post-branchial blood)]/ C_{CO_2tot}(pre-branchial blood)×100.

The injection of saline alone did not cause any changes in the total carbon dioxide content, plasma pH or red cell water content of dorsal or ventral aortic blood (Figs 1 and 2). Taking the slightly different temperatures into account, the dorsal aortic plasma pH of untreated double-cannulated animals was similar to that of fish kept in similar conditions, but cannulated only *via* the dorsal aorta (T=12°C, N=12, pHe=7.754±0.038; mean ± S.E.M.). Thus, double cannulation does not stress the animals more than dorsal aortic

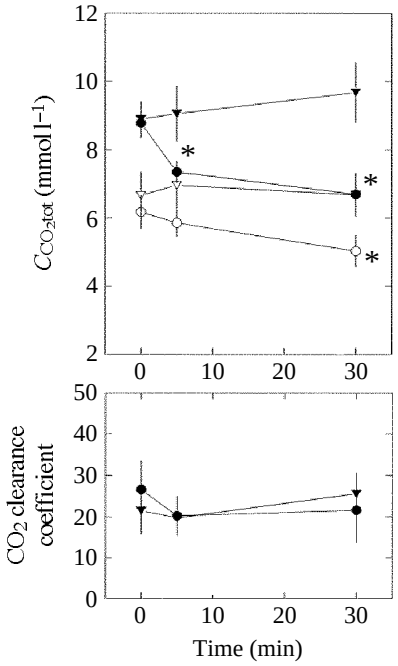

Fig. 2. The total carbon dioxide content [C_{CO_2tot} (mmol l^{-1})] of dorsal (open symbols) and ventral (filled symbols) aortic blood, and the carbon dioxide clearance coefficient {CO_2 clearance coefficient = [C_{CO_2tot}(ventral aorta) − C_{CO_2tot}(dorsal aorta)]/C_{CO_2tot}(ventral aorta)×100} in saline-injected (triangles) and noradrenaline-treated (final nominal concentration approximately 10^{-5} mol l^{-1}; circles) rainbow trout before (0min) and 5 and 30min after the injection. Mean ± S.E.M. are given. For saline-injected fish, N=6; for noradrenaline-injected animals, N=16. Asterisks indicate the statistical significance (P<0.05) of the difference between the mean obtained before injection of noradrenaline and 5 or 30min after the injection. A paired t-test was used for comparisons of the carbon dioxide contents; a Wilcoxon matched-pairs signed-ranks test was used for comparisons of the carbon dioxide clearance coefficient.

cannulation alone. In untreated and saline-injected animals, there was no significant difference between the ventral aortic and dorsal aortic plasma pH. This has been observed before, for example by Kiceniuk and Jones (1977) and Soivio *et al.* (1981). It is probable that plasma pH is not affected significantly during passage through the gills, because a number of protons are liberated from haemoglobin upon oxygenation. The proton release will affect both intra- and extracellular pH and counterbalance any proton extrusion occurring at the gills.

In view of the above, any changes observed in the measured variables must be caused by the 10 μmol l^{-1} noradrenaline. As a result of the catecholamine injection, plasma pH immediately decreased, both in the dorsal and in the ventral aorta (Fig. 1). Simultaneously, the red cell water content increased significantly (Fig. 1). These observations indicate that noradrenaline caused the activation of the sodium/proton exchange across the red cell membrane (Thomas and Motais, 1990; Perry and Thomas, 1991).

The time for the first measurement of total carbon dioxide content of dorsal and ventral aortic blood after catecholamine injection was set as 5min, since at this time the conversion of plasma bicarbonate to carbon dioxide was maximally inhibited by catecholamines *in vitro* (Wood and Perry, 1991) and the increase *in vivo* of carbon dioxide tension of dorsal aortic blood was almost maximal after catecholamine injection (Perry and Thomas, 1991). Noradrenaline caused a reduction of the total carbon dioxide content of both the ventral and the dorsal aortae (Fig. 2). The former change was statistically significant at both 5 and 30min, the latter 30min after the injection of noradrenaline. This finding is opposite to what would have been expected if noradrenaline had caused carbon dioxide retention during the initial minutes after the bolus injection. Provided that noradrenaline does not cause metabolic inhibition, the result suggests that carbon dioxide excretion (per unit time) increases after the injection of noradrenaline into the bloodstream.

Carbon dioxide excretion as a function of time can increase if either the cardiac output or the carbon dioxide clearance coefficient across the gills increases. In the present study, we calculated the carbon dioxide clearance coefficient from the dorsal and ventral aortic total carbon dioxide contents. The reduction of the total carbon dioxide content of whole blood across the gills in the present study was almost exactly the same as the reduction of plasma carbon dioxide content across the gills of resting rainbow trout reported in an earlier study by Nikinmaa and Jensen (1986). There were no statistically significant changes in the carbon dioxide clearance coefficient during the experimental period (Fig. 2). We had a full data set for both dorsal and ventral aortic values from 16 noradrenaline-treated fish. In ten of these, the carbon dioxide clearance coefficient decreased, and in six it increased, 5min after the injection of noradrenaline. These observations indicate that noradrenaline did not impair the efficiency of carbon dioxide excretion per unit volume of blood in the gills. Thus, the reduction of the total carbon dioxide content in blood may reflect a noradrenaline-induced increase in cardiac output. Farrell *et al.* (1986) showed that high concentrations of noradrenaline increase the power output, stroke volume and rate of perfused rainbow trout heart.

It should be pointed out that although the carbon dioxide clearance coefficient, i.e. the efficiency of carbon dioxide removal at the gills, remained practically unchanged throughout the experimental period, the excretion of carbon dioxide per unit volume of blood actually decreased (from approximately 2.7mmol l^{-1} blood to approximately 1.6mmol l^{-1} blood) after the injection of noradrenaline, owing to the reduced carbon dioxide content of ventral aortic blood. Furthermore, it is apparent that throughout the experimental period carbon dioxide excretion at the gills exceeded carbon dioxide production in the tissues, since the total carbon dioxide content of blood was reduced throughout the experiment.

At first sight, the results that catecholamines inhibit the conversion of plasma bicarbonate to carbon dioxide *in vitro* (Wood and Perry, 1991; Perry *et al.* 1991), but that the carbon dioxide clearance coefficient, i.e. the efficiency of carbon dioxide removal at the gills *in vivo*, is not significantly affected, appear to be conflicting. The apparent conflict can, however, be resolved. Catecholamines cause (1) an increase in the proportion of erythrocytes in the blood and (2) an increase in the red cell pH (Nikinmaa,

1982). In addition to plasma bicarbonate, a significant proportion of the excreted carbon dioxide stems from red cell bicarbonate. Erythrocytes contain approximately 15% of the total carbon dioxide content of blood, mainly as bicarbonate, but to a smaller extent also as carboamino compounds (Heming, 1984). The role of red cell bicarbonate in carbon dioxide excretion must be greater than that calculated from the simple distribution of bicarbonate between the plasma and the erythrocytes, because the rate of conversion of red cell bicarbonate to carbon dioxide is not limited by factors (such as anion exchange) that have a half-time approaching the residence time of blood in the gills. Catecholamines increase the red cell number, pH and volume in rainbow trout (Nikinmaa, 1982). This increases the ratio of red cell bicarbonate to total blood bicarbonate and, consequently, the amount of carbon dioxide produced from intra-erythrocytic bicarbonate. Furthermore, an increase in the red cell number, by as much as 30% (Nikinmaa, 1982), will increase the number of anion exchange sites through which plasma bicarbonate can enter the erythrocytes and be converted to carbon dioxide. These factors speed up the conversion of total blood bicarbonate to carbon dioxide. As a consequence, although the conversion of plasma bicarbonate to CO_2 by a constant number of erythrocytes may be slowed down by catecholamines *in vitro* (Wood and Perry, 1991; Perry *et al.* 1991), the conversion of blood bicarbonate *in vivo* may not be.

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