J. exp. Biol. **144**, 507–520 (1989)
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METABOLISM OF ISOLATED FISH GILL CELLS: CONTRIBUTION OF EPITHELIAL CHLORIDE CELLS

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Accepted 23 March 1989

Summary

Gill cell suspensions from freshwater (FW)- and seawater (SW)-adapted teleosts were obtained by density gradient centrifugation. The proportion of chloride cells (CCs) in the mixed cell suspensions was estimated using the fluorescent mitochondrial stain, DASPMEI, and ranged from less than 1 % (FW-adapted tilapia) to approximately 13 % (SW-adapted toadfish). The gill cells displayed relatively high viability based on Trypan Blue exclusion (>75 %), lactate dehydrogenase leakage (<6·5 % h^{-1}), oxygen consumption rates (5–15 μmol g^{-1} cell wet mass h^{-1}) and ATP levels (1–3 μmol g^{-1} cell wet mass). There were no obvious differences between the viability of CCs and the other cell types present.

An initial comparison of gill oxidative metabolism in SW-adapted tilapia (*Oreochromis mossambicus*) and toadfish (*Opsanus beta*) demonstrated that both species oxidized glucose and lactate at substantially greater rates than alanine or oleate. Metabolic rates were significantly higher in toadfish cell suspensions. Kinetic experiments revealed that toadfish gill cells displayed lower values of K_m and higher values of V_m for both lactate and glucose, in comparison to tilapia. The elevated metabolism in toadfish gill cells was correlated with increased activities of the oxidative enzyme citrate synthase and Na^+/K^+-ATPase. The toadfish cell suspensions had a greater proportion of CCs and it is likely that the difference in CC numbers between the two species is the basis for the observed differences in enzyme activities and rates of oxidative metabolism. This idea is supported by the highly significant correlation between Na^+/K^+-ATPase activity (or CC numbers) and rates of lactate oxidation in gill cell suspensions from FW- and SW-adapted tilapia and toadfish, as well as SW-adapted tilapia chronically treated with cortisol to elevate CC numbers.

Although it has been assumed widely that the high metabolic rate of gill tissue reflects, in part, the oxidative demands of the chloride cell, the results of this study provide the first experimental, albeit indirect, evidence for differential rates of metabolism in the various cell types that comprise the gill.

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Key words: Fish, gill, oxidative metabolism, cortisol, chloride cell, pavement cell.

Introduction

The fish gill is a physiologically diverse organ that is involved in gas transfer, ion regulation, acid–base balance and nitrogenous waste elimination. The energy requirements of the latter three processes (especially ion regulation) are thought to be the basis for the exceptionally high oxygen demand of gill tissue (Johansen & Pettersson, 1981; Mommsen, 1984*a*). It has been estimated (see Mommsen, 1984*a*) that the gill may account for as much as 7% of the fish's overall oxygen consumption. It is surprising, therefore, that so few studies have addressed the metabolic aspects of gill function in greater detail. Studies on freshwater rainbow trout (Bilinski & Jonas, 1972; Driedzic & Kiceniuk, 1976; Mommsen, 1984*b*) suggest that the fish gill prefers lactate and glucose as oxidative substrates with less, but substantial, ability to utilize amino acids. In addition, gluconeogenesis is negligible and glycogen stores are low (Mommsen, 1984*a*,*b*). Metabolic studies of fish gill have utilized perfused gills (Johansen & Pettersson, 1981), tissue slices (Bilinski & Jonas, 1972) or tissue homogenates (Mommsen, 1984*b*), and thus have provided no information on the partitioning of metabolism among the various cell types that comprise this morphologically diverse epithelium. The predominant cells in fish gills are pavement cells (PCs), mucus cells and chloride cells (see reviews by Laurent, 1982, 1984). The chloride cell (CC) is probably the site of active transepithelial ionic movements in both freshwater and seawater fishes (Foskett & Scheffey, 1982; Zadunaisky, 1984; Perry & Wood, 1985; Laurent *et al.* 1985; Avella *et al.* 1987; Perry & Flik, 1988). Thus, it has been widely assumed that the mitochondria-rich CCs (Laurent, 1984) are metabolically more active than the other cell types, although there are no direct data to support this idea. The initial objective of the present study, therefore, was to obtain enriched fractions of CCs and PCs from marine teleosts in order to compare their metabolic rates and substrate preferences. We were unable substantially to purify these cell types but we could consistently prepare mixed cell suspensions in which the density of CCs was quantifiable. Preliminary experiments demonstrated significantly different rates of oxidative metabolism in mixed cell suspensions from two seawater-adapted teleosts that appeared to be related to differences in CC density. Thus, further experiments were designed to test the hypothesis that CCs possess higher metabolic rates than other gill cell types and that species- or environment-dependent differences in gill metabolism are related to differential abundance of branchial chloride cells.

Materials and methods

Experimental animals and acclimation regimes

Gulf toadfish, *Opsanus beta* [mean mass = $181 \cdot 7 \pm 37 \cdot 0$ g (±S.E.); experimental $N = 7$] were obtained by trawl from Biscayne Bay (Florida) by local fishermen in November 1987. Toadfish were maintained outdoors in concrete aquaria supplied with flowing sea water at 25–26°C under a natural photoperiod. They were not fed and were used within 1 week of capture. Freshwater tilapia (*Oreochromis*

mossambicus) were purchased from Sunshine Aquaculture (Homestead, Florida). Some were maintained in sea water for approximately 1 year (mean mass = 290·2 ± 13·7 g; experimental $N = 7$), whereas others (mean mass = 393 ± 41·7 g, experimental $N = 6$) were kept in dechlorinated City of Miami tap water ($[Na^+] = 0{\cdot}96\,mmol\,l^{-1}$; $[Cl^-] = 0{\cdot}99\,mmol\,l^{-1}$; $[K^+] = 0{\cdot}05\,mmol\,l^{-1}$; $[Ca^{2+}] = 0{\cdot}50\,mmol\,l^{-1}$). In addition, a separate group of seawater-adapted tilapia (mean mass = 256·6 ± 26·4 g; experimental $N = 6$) were given daily intramuscular injections of cortisol (hydrocortisone hemisuccinate disodium salt; Sigma) dissolved in 0·6 % NaCl at a dose of $4\,mg\,kg^{-1}$ for a 7-day period to induce branchial chloride cell proliferation (see Perry & Wood, 1985). All tilapia were maintained indoors in large circular fibreglass tanks supplied with the appropriate acclimation water at 25 °C. The photoperiod was 12 h light: 12 h dark. Seawater-adapted tilapia were fed a diet of commercial salmon pellets (Rangen Company; Buhl, Idaho) twice weekly, whereas the freshwater-adapted tilapia were not fed and were used within 1 week after their arrival.

Isolation of gill cells and viability criteria

A blood sample (approximately 1·0 ml) was withdrawn from the caudal artery/vein, centrifuged, and the plasma stored at −70 °C for subsequent analyses of lactate and glucose levels. The fish then was injected in the caudal artery/vein with 5000 units of heparin (sodium salt, Sigma) dissolved in 0·6 % NaCl ($5000\,units\,ml^{-1}$). After about 5 min, the fish was decapitated and the bulbus arteriosus catheterized using flexible polyethylene tubing (PE 90; Clay Adams). The head was immersed in water and the gills perfused *via* the catheter at a constant pressure of 5·89 kPa with Ca^{2+}- and Mg^{2+}-free balanced salt solution (BSS; Hootman & Philpott, 1978) for approximately 5 min. This procedure was usually sufficient to clear the gill arches of red blood cells (RBCs). Occasionally, considerable numbers of red cells remained trapped in the gills, reflecting poor perfusion of the gills with Ca^{2+}-, Mg^{2+}-free BSS, and therefore these gills were discarded. The branchial basket was excised and placed in 50 ml of ice-cold Ca^{2+}-, Mg^{2+}-free BSS and gently stirred on ice for 30 min. The gill arches were placed on an ice-cold glass plate and the tissue was removed by carefully scraping the arches with the edge of a glass microscope slide. This crude homogenate then was combined with the original 50 ml of Ca^{2+}-, Mg^{2+}-free BSS, filtered through nylon mesh (mesh sizes = 253 and 73 μm), and the filtrate was centrifuged at 150 g for 5 min in a clinical bench-top centrifuge. The pellet was resuspended in 5 ml of Ca^{2+}-, Mg^{2+}-free BSS. In preliminary experiments, the resuspended pellet was layered over a discontinuous Ficoll (Sigma) gradient according to Hootman & Philpott (1978). However, in all experiments yielding metabolism data, the resuspended pellet was mixed with a Percoll (Sigma) solution with an initial density of $1{\cdot}07\,g\,ml^{-1}$ (obtained by diluting stock Percoll ($1{\cdot}13\,g\,ml^{-1}$) with an appropriate volume of concentrated Ca^{2+}-, Mg^{2+}-free BSS). Subsequent centrifugation produced a continuous gradient from 1·02 to $1{\cdot}13\,g\,ml^{-1}$. For both Ficoll and Percoll, the samples were centrifuged at 20 000 g for 45 min at 4 °C using a

Sorvall RC5B centrifuge. After centrifugation, discrete layers of cells were obvious and were collected by aspiration using a 5 ml pipette. The cells were washed in 20 ml of Ca^{2+}-, Mg^{2+}-free BSS and centrifuged at 500 *g* for 5 min to remove gradient material. Finally, the pellet was resuspended in 3 ml of appropriate metabolic medium [identical to that used for toadfish hepatocytes with fatty acid-free bovine serum albumin (4 %), Walsh, 1987] to yield cell suspension concentrations between 10 and 69 mg cell wet mass ml^{-1} (mean concentration = 35·8 ± 3·6 mg cell wet mass ml^{-1}). Cell wet masses were determined by weighing cell pellets after centrifugation of cell suspension samples in microcentrifuge tubes (1·5 ml) at 13 000 *g* for 1 min.

Cells were stained with 0·2 % Trypan Blue and examined microscopically using a haemocytometer for percentage of viable cells (i.e. excluding Trypan Blue). A portion of the cell suspension was fixed in 0·6 mol l^{-1} $HClO_4$ and stored at −70 °C for later determination of ATP levels (Walsh, 1987). Finally, cell suspensions were centrifuged for 1 min at 13 000 *g* at the beginning and end of an experiment. Lactate dehydrogenase (LDH) activity was monitored in the supernatant (Walsh *et al.* 1987) and in a lysate of the pellet and the rate of leakage of LDH from the cells during the experiment was calculated.

Measurements of metabolism and enzyme assays

Rates of oxidative metabolism were determined by measuring the production of $[^{14}C]CO_2$ (and then calculating absolute values of CO_2 production) after incubating the cell suspensions (600 μl) with $[^{14}C]$-labelled substrate, as described in detail for fish hepatocytes (Walsh *et al.* 1988). The oxidative substrates used were $[6\text{-}^{14}C]$glucose (2 mmol l^{-1}), $[^{14}C]$lactate (2 mmol l^{-1}), $[^{14}C]$alanine (2 mmol l^{-1}) and $[^{14}C]$oleate (0·25 mmol l^{-1}). All radiochemicals (New England Nuclear; Boston, MA) except glucose were uniformly labelled (see above) and were diluted to a final specific activity of 0·25 μCi μmol^{-1}. Cells were incubated for 1 h either with a single substrate or with multiple substrates in competition experiments designed to provide information on substrate preference (see Mommsen, 1984*b*). Total oxygen consumption rates were measured using a sealed small-volume (800 μl) metabolic chamber fitted with a Clarke-type oxygen electrode and YSI (model 53) oxygen meter.

Rates of gluconeogenesis were determined by monitoring the production of $[^{14}C]$glucose from radiolabelled alanine and/or lactate (Walsh *et al.* 1988). The activities of citrate synthase (an indicator of Krebs cycle activity) and Na^+/K^+-ATPase were measured on cell pellet lysates according to Walsh *et al.* (1987) and Bonting & Caravaggio (1963), respectively. The liberated inorganic phosphate in the ATPase assay was measured using a commercial spectrophotometric assay kit (Sigma).

Quantification of chloride cell numbers

Chloride cell numbers were quantified in the mixed gill cell suspensions using the fluorescent mitochondrial stain DASPMEI (dimethyl-aminostyrylmethylpyri-

diniumiodide) as previously applied to fish opercular epithelial CCs (Karnaky *et al.* 1984). Cells were incubated in darkness for 30 min with 25 μmol l^{-1} DASPMEI, washed in suspension medium to remove excess stain, and then examined on a haemocytometer using phase contrast or epifluorescence microscopy (Leitz Laborlux D microscope).

Results

Density gradient centrifugation using a discontinuous Ficoll gradient yielded a cell layering pattern essentially identical to that previously reported for pinfish gill (Hootman & Philpott, 1978). The lowest discrete layer of cells (termed band C by Hootman & Philpott) contained approximately 70 % CCs as determined by DASPMEI staining. The overall yield, however, in this layer (about 25 mg cell wet mass) was insufficient to permit multiple metabolic experiments and therefore this approach was abandoned. All subsequent experiments were performed on cells obtained using a continuous Percoll gradient. Essentially, two zones of cells were present after centrifugation, a layer approximately 1 cm from the top of the gradient and a diffuse layer approximately 1 cm from the bottom of the gradient. The lower layer contained erythrocytes, cellular debris, clumps of cells and damaged cells. The upper layer contained a mixture of CCs, presumptive PCs, debris, damaged cells and the occasional erythrocyte. The CCs were easily identifiable using DASPMEI (Figs 1, 2). Under the phase contrast microscope, the CCs were distinguishable by a distinct opaque cellular membrane (Figs 1, 2). The CCs often, but not always, appeared granulated and were, in general, larger than the presumptive PCs. The CCs ranged between 10 and 15 μm in diameter whereas the presumptive PC usually were less than 10 μm in diameter. It was obvious that cell size and degree of granulation were not useful indicators of cell type. For this reason, the proportion of CCs in the mixed cell populations was quantified solely on the basis of DASPMEI staining. The proportion of CCs in the cell suspensions varied according to species and acclimation medium (FW *versus* SW). The cell suspensions prepared from FW-adapted tilapia displayed the fewest CCs (<1 %), whereas the SW-adapted toadfish displayed the greatest proportion (approximately 13 %). The CCs in suspension did not resemble the CCs from intact gill or opercular epithelia. The basolateral membrane, with its characteristic infoldings and invaginations, was not identifiable in cell suspensions. Indeed, there was no evidence of distinct apical and basolateral membranes.

The criteria used to assess gill cell suspension viability are displayed in Table 1. Trypan Blue exclusion was in excess of 75 % and LDH leakage was low (<6·5 % h^{-1}). The ATP contents of the gill cells were stable in all experimental groups except in the cortisol-injected SW-adapted tilapia. In this group, a significant decrease in [ATP] occurred over the 1-h incubation period.

Oxygen consumption in the presence of mixed substrate (lactate, glucose and alanine) was substantially higher (2·9-fold) in toadfish gill cells than in tilapia gill cells (Table 2). Gill cells from both species displayed higher metabolic rates with

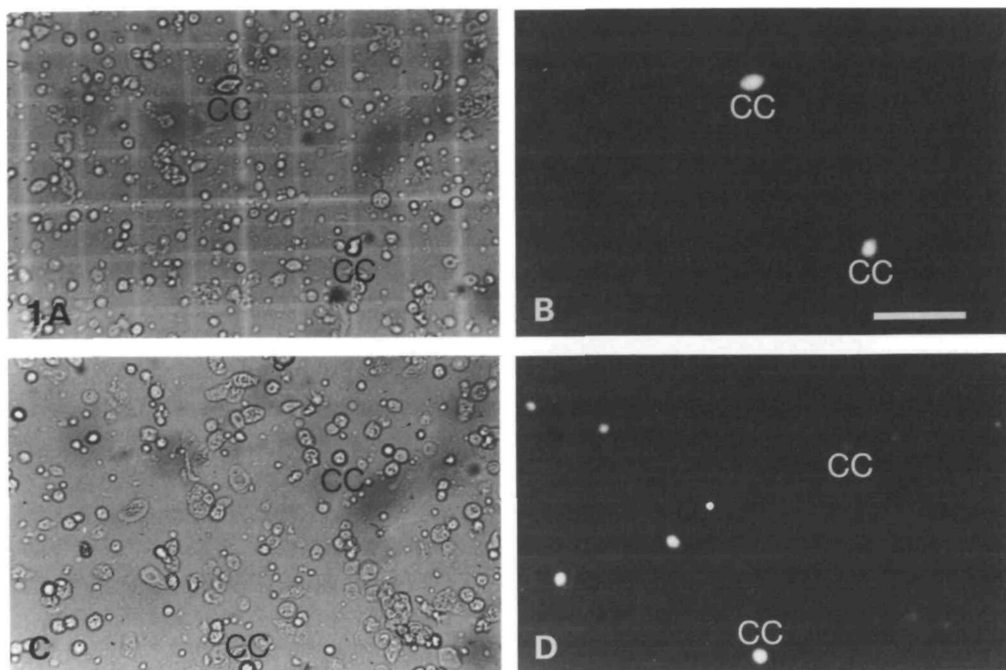

Fig. 1. The microscopic appearance of DASPMEI-stained cells from gill cell suspensions of seawater (SW)-adapted (A,B) tilapia (*Oreochromis mossambicus*) and (C,D) toadfish (*Opsanus beta*) as viewed under phase contrast (A,C) or epifluorescence (B,D). CC, chloride cell. Scale bar, 100 μm.

Table 1. *Viability of gill cell suspensions used in metabolism studies*

Fish	Trypan Blue exclusion (% unstained cells)	LDH leakage (% h^{-1})	[ATP]i* (μmol g^{-1} cell wet mass)	[ATP]f† (μmol g^{-1} cell wet mass)
SW tilapia (6)	89 ± 3·9	6·5 ± 4·1	3·1 ± 0·7	3·4 ± 0·6
SW Cortisol-injected tilapia (5)	84 ± 3·7	2·2 ± 1·2	1·1 ± 0·2	0·6 ± 0·1**
FW tilapia (6)	76 ± 2·7	6·2 ± 2·8	1·1 ± 0·2	1·8 ± 0·2
SW toadfish (7)	78 ± 3·4	ND	2·2 ± 0·9	2·0 ± 0·5

Values shown are means ± 1 S.E., *N* numbers are indicated in parenthesis.

*[ATP]i is the initial concentration of ATP in the cell suspension just prior to the 1-h incubation period with [^{14}C]-labelled substrate(s).

† [ATP]f is the final concentration of ATP in the cell suspension after the 1-h incubation period with [^{14}C]-labelled substrate(s).

ND, non-detectable.

** Significantly different from [ATP]i ($P \leq 0{\cdot}05$; paired Student's *t*-test).

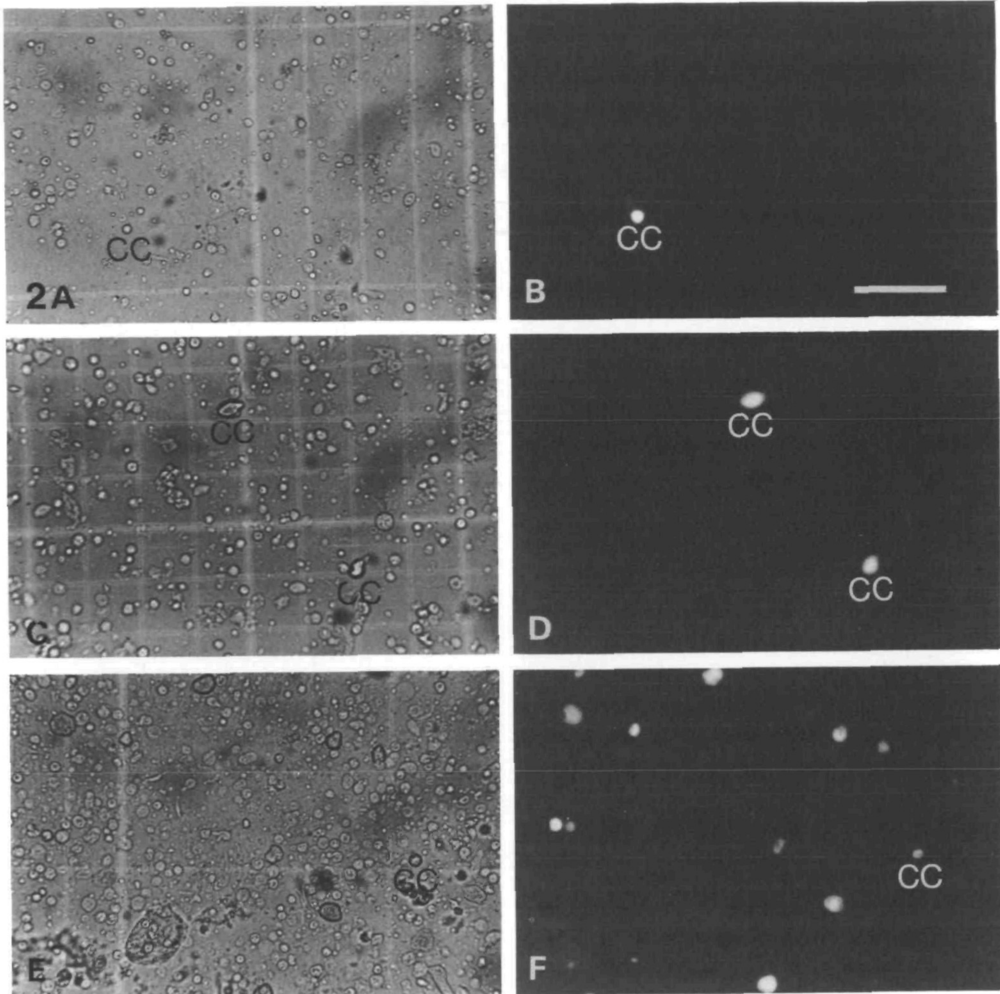

Fig. 2. The microscopic appearance of DASPMEI-stained cells from gill cell suspensions of (A,B) freshwater (FW)-adapted tilapia, (C,D) seawater (SW)-adapted tilapia, and (E,F) SW-adapted tilapia chronically treated with cortisol as viewed under phase contrast (A,C,E) or epifluorescence (B,D,F). CC, chloride cell. Scale bar, 100 μm.

glucose or lactate as substrate than with alanine or oleate (oleate was not tested in the toadfish). In substrate preference experiments, however, toadfish gill cells appeared to exhibit a preference for glucose. Glucose markedly inhibited lactate metabolism in toadfish cells and indeed lowered the rate of lactate utilization to the level of tilapia (Table 2). In the presence of competing lactate, glucose oxidation was depressed, but remained significantly greater than in tilapia (Table 2). Kinetic analyses of lactate and glucose oxidation (Fig. 3) confirmed that toadfish gill cells had a lower K_m and a higher V_m for both lactate and glucose than

Table 2. *Oxidative metabolism in gill cell suspensions from seawater-adapted tilapia* (Oreochromis mossambicus) *or toadfish* (Opasanus beta)

Substrate(s)	Tilapia				Toadfish			
	Carbon dioxide production (μmol g^{-1} cell wet mass h^{-1})							
	No additions	+ Lactate	+ Glucose	+ Alanine	No additions	+ Lactate	+ Glucose	+ Alanine
Lactate (6)	0·50 ± 0·06	–	0·36 ± 0·06	0·47 ± 0·06	1·11 ± 0·26*	–	0·59 ± 0·15^{NS}	0·91 ± 0·22*
Glucose (6)	0·36 ± 0·06	0·13 ± 0·03	–	0·31 ± 0·07	2·30 ± 0·85*	0·83 ± 0·36*	–	2·21 ± 0·83*
Alanine (6)	0·14 ± 0·04	0·08 ± 0·02	0·09 ± 0·03	–	0·08 ± 0·01	0·07 ± 0·01	0·07 ± 0·01	–
Oleate (6)	0·05 ± 0·03	–	–	–	–	–	–	–
	Oxygen consumption (μmol g^{-1} cell wet mass h^{-1})							
Lactate; Glucose; Alanine (6)	4·82 ± 0·94	–	–	–	13·82 ± 2·49*	–	–	–

Values shown are means ± 1 S.E.; N numbers are indicated in parenthesis.

* Significantly different from corresponding value in tilapia ($P \leqslant 0·05$; unpaired Student's t-test).

NS, non-significant.

All substrates were added to a final concentration of 2 mmol l^{-1}, except oleate which was 0·25 mmol l^{-1}.

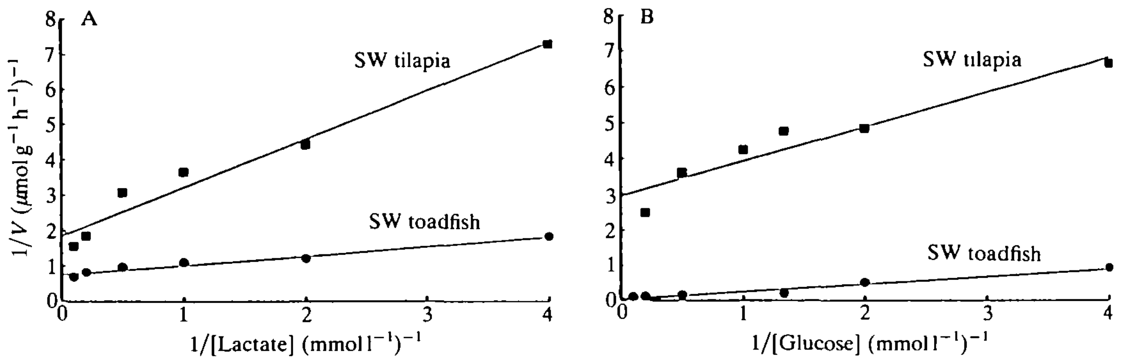

Fig. 3. Lineweaver–Burke plots demonstrating the substrate-dependency of oxidative metabolism on (A) lactate and (B) glucose in gill cell suspensions from SW-adapted tilapia (■) and toadfish (●). The K_m values for lactate are 0·73 mmol l^{-1} [tilapia; $1/V = 1{\cdot}36(1/[\text{lactate}]) + 1{\cdot}86$; $r = 0{\cdot}982$] and 0·35 mmol l^{-1} [toadfish; $1/V = 0{\cdot}26(1/[\text{lactate}]) + 0{\cdot}76$; $r = 0{\cdot}981$]; V_m values for lactate are 0·54 μmol g^{-1} h^{-1} (tilapia) and 1·32 μmol g^{-1} h^{-1} (toadfish). The K_m values for glucose are 0·33 [tilapia; $1/V = 0{\cdot}96(1/[\text{glucose}]) + 2{\cdot}96$; $r = 0{\cdot}951$] and 0·17 [toadfish; $1/V = 0{\cdot}21(1/[\text{glucose}]) + 0{\cdot}04$; $r = 0{\cdot}983$]; the V_m values for glucose are 0·42 μmol g^{-1} h^{-1} (tilapia) and 27·9 μmol g^{-1} h^{-1} (toadfish).

tilapia. The differences in glucose kinetic parameters between the two species were more pronounced than the differences in lactate kinetic parameters (Fig. 3).

The plasma concentrations (in mmol l^{-1}) of lactate and glucose in toadfish were 0·65 ± 0·11 and 1·67 ± 0·27, respectively, and the corresponding values in SW-adapted tilapia were 1·64 ± 0·35 and 4·19 ± 0·35. These concentrations are above the K_m values for lactate and glucose in both species (see Fig. 3) and thus both are potential oxidative substrates, *in vivo*.

Gluconeogenic rates were non-detectable in toadfish gill cell suspensions. In tilapia, gluconeogenesis was detectable using alanine only. The rate of glucose production from alanine was 0·058 ± 0·025 nmol g^{-1} cell wet mass h^{-1} ($N = 6$), or approximately 400-fold lower than oxidative rates.

Noting the large difference in gill oxidative metabolism between the two species, we attempted to determine the physiological basis for this difference. The elevated rates of oxidative metabolism in toadfish gill cells were associated with higher activities of citrate synthase and Na^+/K^+-ATPase (Table 3). The higher activity of these enzymes in toadfish gill suspensions could reflect increased enzyme levels in all of the cell types or, alternatively, an increased proportion of cells (e.g. CCs) with high levels of these enzymes. The latter is supported by the higher density of CCs in toadfish gill cell suspensions than in those of tilapia (Fig. 1).

The differences in oxidative metabolism between gill cell suspensions from SW-adapted toadfish and tilapia appeared to be correlated with numbers of CCs. In an attempt to test this relationship more firmly, the density of CCs in gill cell suspensions was manipulated experimentally. The use of FW-adapted tilapia and

Table 3. *Enzyme activities in gill cell suspensions from seawater-adapted-tilapia (*Oreochromis mossambicus*) and toadfish (*Opsanus beta*)*

	Tilapia	Toadfish
Citrate synthase	$0{\cdot}02 \pm 0{\cdot}03$	$0{\cdot}05 \pm 0{\cdot}01$*
(nmol min^{-1} mg^{-1} protein)	(8)	(7)
Na^+/K^+-ATPase	$3{\cdot}98 \pm 0{\cdot}71$	$12{\cdot}2 \pm 1{\cdot}82$*
(μmol Pi h^{-1} mg^{-1} protein)	(8)	(7)
LDH	$24{\cdot}4 \pm 5{\cdot}4$	$24{\cdot}0 \pm 6{\cdot}10$
(μmol min^{-1} g^{-1} wet mass)	(8)	(5)

* Significantly different from corresponding value in tilapia ($P \leq 0{\cdot}05$; unpaired Student's *t*-test.

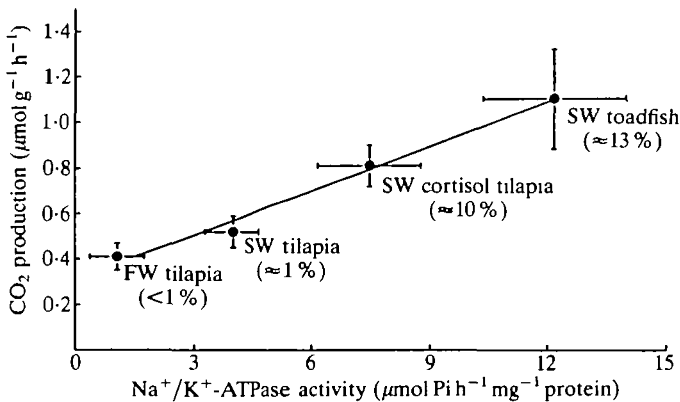

Fig. 4. Correlation between Na^+/K^+-ATPase activity and CO_2 production [CO_2 production $= 0{\cdot}064(Na^+/K^+\text{-ATPase activity}) + 0{\cdot}31$; $r = 0{\cdot}994$] from lactate in gill cell suspensions displaying a wide range of chloride cell (CC) numbers. The percentage of CCs in the cell suspensions is indicated in parenthesis.

SW-adapted tilapia chronically treated with cortisol produced a marked range of CC densities in gill cell suspensions, as indicated by specific DASPMEI staining (Fig. 2; see also Fig. 4). The data represented in Fig. 4 demonstrate that Na^+/K^+-ATPase (and CC density) co-vary with lactate oxidation and support the hypothesis that the differences in metabolism between the toadfish and tilapia are related to the abundance of gill epithelial CCs.

Discussion

Methodology

Although it was possible to isolate relatively enriched fractions of CCs (approx. 70 %) using the technique of Hootman & Philpott (1978), the yield was insufficient

to permit metabolic experiments. This result probably should have been expected, given the relatively large quantities of cells required for multifractional metabolic studies employing radiolabelled substrates and the small percentage of the total gill epithelium that consists of CCs (see reviews by Laurent, 1982, 1984). Thus, we were unable to compare metabolic rates of enriched CC suspensions with presumptive PC suspensions (the PCs are termed presumptive because, unlike the CCs, there is no specific marker that can be applied for identification). Instead, we utilized a simpler continuous gradient centrifugation technique that yielded a mixed gill cell population in which the density of CCs was easily quantified. These gill cell suspensions had high viability when compared with other gill cell suspensions obtained using discontinuous density gradients (Kamiya, 1972; Sargent *et al.* 1975; Hootman & Philpott, 1978) but had lower viability (based on Trypan Blue exclusion) than eel gill cell suspensions obtained by velocity sedimentation (Naon & Mayer-Gostan, 1983).

In the present study, the initial ATP levels in the gill cells were similar to those reported for homogenized rainbow trout filaments (Mommsen, 1984*b*). Oxidation rates were also similar to values reported previously for perfused gill preparations of trout (Wood *et al.* 1978) and cod (Johansen & Pettersson, 1981). Furthermore, oxidation rates varied according to type and/or concentration of substrate. Thus, unlike the gill cell suspensions obtained by discontinuous density gradient centrifugation (Sargent *et al.* 1975; Hootman & Philpott, 1978), the cells in the present study were clearly viable metabolically. This obviously is the most pertinent criterion for judging the usefulness of a particular preparation in studies of metabolism. We believe, therefore, that isolated fish gill cell suspensions offer great potential in the further characterization of the metabolic properties of the fish gill.

It is unclear why CO_2 production rates were markedly lower than rates of O_2 consumption (Table 2), although it is likely that CO_2 production calculated on the basis of $[^{14}C]CO_2$ production from a single labelled substrate will always underestimate total oxidative metabolism, given the probability of endogenous substrate oxidation. Furthermore, O_2 consumption was measured under distinctly different conditions (in the presence of combined glucose, lactate and alanine each at a concentration of $2\,mmol\,l^{-1}$) from those used in the CO_2 production experiments.

Metabolism of gill cell suspensions: contribution of the epithelial chloride cell

The results of this study demonstrate that both lactate and glucose are important oxidative substrates for gill cells from SW-adapted toadfish and tilapia. These results concur with results obtained using gill filaments of FW-adapted rainbow trout (Mommsen, 1984*b*). In the absence of appreciable gill cell gluconeogenesis (see also Bilinski & Jonas, 1972; Knox *et al.* 1980; Mommsen, 1984*b*), the source of glucose *in vivo* must be the blood perfusing the gills. Similarly, the highly aerobic gill tissue is unlikely to produce sufficient lactate for oxidative metabolism and thus the origin of the oxidized lactate *in vivo* is presumably also venous blood.

Indeed, the fact that the levels of lactate (Driedzic & Kiceniuk, 1976; Soivio *et al.* 1981; Mommsen, 1984*b*) and glucose (Mommsen, 1984*b*) are higher in venous than in arterial blood in resting rainbow trout indicates that these two substrates are extracted by the gill cells and used for oxidative metabolism. It is difficult to assess the relative importance of lactate and glucose to gill cell oxidative metabolism in the intact animal based on the results of our *in vitro* experiments. The measured blood lactate and glucose levels are probably representative of the resting fish (subsequent measurements of blood lactate and glucose concentrations in chronically catheterized resting toadfish were 0·4 and 1·3 $mmol\,l^{-1}$, respectively; S. F. Perry & P. J. Walsh, unpublished data). Therefore, given the kinetic relationships (Fig. 3) and competition data (Table 2), it is likely that glucose is the preferred metabolic fuel at rest in both tilapia and toadfish. The relationship might change, however, as physiological state changes. For example, after exercise in toadfish, plasma [lactate] increases to approximately 5 $mmol\,l^{-1}$ at 1·5 h, whereas plasma [glucose] rises steadily to nearly 3 $mmol\,l^{-1}$ at 8 h post-exercise (S. F. Perry & P. J. Walsh, unpublished data) which might lead to changes in substrate utilization. Moreover, it has been suggested that the two substrates might support different physiological processes (Mommsen, 1984*a*,*b*). Indeed, this idea is supported by the results of the present study. Toadfish gill cell suspensions displayed greater rates of metabolism in the presence of both lactate or glucose than tilapia cells. The increase in oxidation from glucose, however, was much more pronounced than the increase in oxidation from lactate (Table 2, Fig. 3). The toadfish gill cell suspensions had a greater proportion of CCs and thus it is conceivable that the CC prefers glucose as an oxidative substrate and that this may reflect the predominant role of the CC in active transepithelial ion movements (see below).

A variety of studies suggest that the chloride cell is the site of active ion transporting processes involved in ionic regulation in freshwater and marine fishes (Girard & Payan, 1980; Foskett & Scheffey, 1982; Zadunaisky, 1984; Perry & Wood, 1985; Laurent *et al.* 1985; Avella *et al.* 1987; Perry & Flik, 1988). Ionic regulation, especially in marine fishes, can account for a substantial portion of a fish's energy budget (e.g. Febry & Lutz, 1987) owing to the hydrolysis of ATP by the various ion-transport ATPases. These include Na^{+}/K^{+}-ATPase, high-affinity Ca^{2+}-ATPase and perhaps the poorly-defined Cl^{-}/HCO_3^{-}-ATPase (see review by De Renzis & Bornancin, 1984). These ion-transport ATPases are present in both the pavement and chloride cells, although they are substantially more concentrated in the chloride cells (Kamiya, 1972; Sargent *et al.* 1975; Hootman & Philpott, 1978; Naon & Mayer-Gostan, 1983). For this reason, it has been assumed that the CCs are metabolically more active that the PCs. The results of this study demonstrate, albeit indirectly, that CCs do indeed have higher metabolic rates than other gill cell types, and that the overall metabolic rate of the gill cell population closely reflects the number of CCs present. Increases in CC density (as measured directly by DASPMEI staining) and in its enzymatic correlate, Na^{+}/K^{+}-ATPase, were closely linked to oxidative metabolism both within a single

species (*O. mossambicus*) adapted to FW (relatively low density of CCs) or SW (relatively high density of CCs) environments and in two SW species displaying varied abundances of CCs. It is interesting that the activity of an anaerobic enzyme (LDH) did not vary in cell suspensions with different aerobic metabolic rates and CC densities.

In addition to demonstrating a link between the rate of gill cell metabolism and CC density, the present study identifies the potential of isolated cells *in vitro* for further study of fish gill metabolism.

This study was supported financially by a NSERC of Canada operating grant to SFP and a NSF grant (DCB-8608728) and an NIH grant (ES-4361) to PJW. We gratefully appreciate the technical assistance of Dr Pat Blackwelder, Vicki Credle and Naomi Chasens for microscopy. Jimbo Luznar provided the toadfish specimens. We are grateful to Dr Liz Clarke and the RSMAS Experimental Fish Hatchery for providing aquarium facilities and Dr Larry Brand for the use of his epifluorescence microscope. We thank Dr. T. W. Moon for critical comments on the manuscript.

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