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EFFECTS OF WHEAT GERM AGGLUTININ AND COLCHICINE ON MICROTUBULES OF THE MITOCHONDRIA-RICH CELLS AND Ca^{2+} UPTAKE IN TILAPIA (*OREOCHROMIS MOSSAMBICUS*) LARVAE

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Summary

The organization of the microtubules and actin filaments in the gills of tilapia (*Oreochromis mossambicus*) larvae was revealed by confocal microscopy. The fluorescence intensity of the microtubules in the gills was increased by adding wheat germ agglutinin (WGA, 40 ng ml^{-1}) to the ambient water for 30 min, but the staining pattern of the actin filaments was not changed. The fluorescence intensity of the microtubules in the gills was decreased by treatment with the microtubule-disrupting reagent colchicine at 0.2 mmol l^{-1} for 4 h. WGA treatment concurrently raised Ca^{2+} influx rates, and the increase was particularly large when the larvae were kept in water with extremely low Ca^{2+} levels ([Ca^{2+}]=0.002 mmol l^{-1}). Colchicine treatment, in contrast, reduced the Ca^{2+} influx rate. These results indicate that the microtubule network in tilapia gills, particularly in mitochondria-rich cells, could play a critical role in the uptake of Ca^{2+} in tilapia larvae.

Key words: mitochondria-rich cell, gill, fish, cytoskeleton, fresh water, confocal microscopy, colchicine, wheat germ agglutinin, ionic regulation, tilapia, *Oreochromis mossambicus*.

Introduction

The gill is the major organ specialized for ionic regulation in fish. In gills, the mitochondria-rich (MR) cell, also known as the chloride cell, is a maturely differentiated cell type and is believed to be the main site of ion uptake or extrusion (Hwang and Hirano, 1985; Hwang *et al.* 1989; Goss *et al.* 1993; Li *et al.* 1997; Perry, 1997). Many studies have shown that the MR cell of fish gills is the main site of Ca^{2+} uptake, especially in freshwater fish (Flik *et al.* 1985; Ishihara and Mugiya, 1987; Marshall *et al.* 1992; McCormick *et al.* 1992; Flik and Verbost, 1995; Hwang *et al.* 1994, 1996). Our previous study (Tsai and Hwang, 1998) demonstrated that there are numerous binding sites for wheat germ agglutinin (WGA) in the apical area of certain MR cells in tilapia gills. In addition, the number of WGA-positive MR cells increases in the gills of tilapia adapted to a comparatively low-[Ca^{2+}] medium. Accordingly, the WGA binding sites at the apex of the MR cells were assumed to be functional in the absorption of Ca^{2+} from the ambient water.

Additional evidence is needed to prove that the WGA binding sites are involved in Ca^{2+} uptake. Moreover, the identity of the components in the MR cells that interact with these sites is intriguing. Several studies have indicated that WGA treatment of frog urinary bladder cells (Favard *et al.* 1989), cultured chick embryo fibroblasts (Arena *et al.* 1990), red blood cells (Danilov and Cohen, 1989; Lin and Huestis, 1995) and intestinal cells (Sjolander and Magnusson, 1988) causes changes in cell shape and in the organization of cytoskeletal proteins. WGA may interact with certain glycoproteins in the membrane junctions of the cytoskeletal network, eliciting reorganization of actin filaments or microtubules and resulting in modulation of specific cellular events.

It is thus possible that the WGA binding sites at the apex of MR cells are related to the cytoskeletal structure. Furthermore, if the WGA binding sites are involved in the uptake of Ca^{2+}, as our previous study implied, then the Ca^{2+} influx rate should be altered following the addition of free WGA molecules to the ambient water. The present study, therefore, was aimed at revealing the organization of the microtubules and actin filaments in the gill epithelial cells of tilapia larvae. The effects of WGA and colchicine on the cytoskeletal network were examined. We demonstrate that the organization of microtubules in the MR cells was altered by these two reagents and also that the Ca^{2+} influx rate was modulated.

Materials and methods

Animals

Mature adult tilapia (*Oreochromis mossambicus* Peters) from the Tainan branch of the Taiwan Fisheries Research Institute were reared in circulating Taipei local fresh water at 27 °C and under a 12 h:12 h L:D photoperiod. Fertilized eggs

were incubated under these same conditions. Larvae 5 days after hatching, approximate mass 0.005–0.008 g, and juveniles approximately 1 month old, mass 2.0–2.5 g, were used in experiments. Three media varying in Ca^{2+} level were prepared by mixing $CaSO_4$, $MgSO_4$, NaCl, K_2HPO_4 and KH_2PO_4 with double-deionized water (Milli-RO60, Millipore, USA) for the Ca^{2+} influx experiments. The compositions of Taipei local fresh water and the three artificial media are listed in Table 1.

Staining of microtubules, actin filaments and Na^+/K^+-ATPase in gills of tilapia

Fresh gills were excised from tilapia larvae and juveniles. For microtubule fixation (Parczyk *et al.* 1989), the gills were first fixed with methanol at −20 °C for 10 min and then with acetone at −20 °C for another 10 min. The whole gills of larvae were then stained as described in the next paragraph. To obtain a clear image of the microtubules in the gill, the gills of juveniles were first immersed in 30 % sucrose plus 1 % gum arabic, and 10 μm frozen cross sections were then cut using a cryostat (Bright, UK) and attached to slides coated with poly-L-lysine (Sigma). For actin filament fixation, the gills of larvae and juveniles were fixed with phosphate-buffered 4 % paraformaldehyde plus 2 % glutaraldehyde for 1 h at 4 °C. Similarly, to obtain a clear cross image of the actin filaments in the gills, 10 μm frozen cross sections of the juveniles gills were also prepared as described above.

To stain microtubules, the methanol/acetone-fixed gills of tilapia larvae or methanol/acetone-fixed frozen sections of juvenile gills were rinsed with phosphate-buffered saline (PBS: NaCl, 140 mmol l^{-1}; Na_2HPO_4, 8 mmol l^{-1}; KH_2PO_4, 1.5 mmol l^{-1}; KCl, 2.7 mmol l^{-1}; pH 7.4) and then incubated in PBS containing 0.5 % Triton-X100 for 30 min. All the reactions were performed at room temperature (25–27 °C). After several rinses with PBS, the gills were incubated with 10 % normal goat serum (Jackson Immunoresearch Laboratories, Inc., USA) in PBS for 30 min, in anti-α-tubulin monoclonal antibody (Sigma, diluted 1:100) for 2 h, and in goat anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) (Jackson Immunoresearch Laboratories, Inc., USA, diluted 1:100) for 1 h. Double-staining for Na^+/K^+-ATPase was carried out to localize the MR cells, which have high concentrations of this enzyme (Hootman and Philipott, 1979; Kultz and Jurss, 1993). This was achieved by an additional incubation with polyclonal Ab-TG3 antiserum against Na^+/K^+-ATPase (Hwang *et al.* 1998), diluted 1:100, for 1 h and followed by incubation with goat anti-rabbit IgG conjugated with tetramethylrhodamine isothiocyanate (TRITC) (Jackson Immunoresearch Laboratories, Inc., USA, diluted 1:100) for 1 h. All the antibodies were diluted in PBS containing 3 % bovine serum albumin (Sigma, USA). Between each step, three PBS washings, each lasting 5 min, were performed.

Actin filament staining was carried out according to Adams and Pringle (1991). The glutaraldehyde/paraformaldehyde-fixed gills of larvae or glutaraldehyde/paraformaldehyde-fixed frozen sections of juvenile gills were first rinsed with PBS and then incubated with PBS containing 0.5 % Triton X-100 for 30 min. All reactions were performed at room temperature. After several rinses with PBS, the gills were stained with phalloidin–TRITC (Sigma, USA; 15 nmol l^{-1}, diluted in PBS) for 30 min. Double-staining for Na^+/K^+-ATPase was also conducted as described above, but the secondary antibody was replaced by a goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC) (Jackson Immunoresearch Laboratories, Inc., USA, diluted 1:100).

Confocal laser scanning microscopy

Observations were made using a Bio-Rad MRC 1000 confocal laser scanning microscope attached to a Zeiss microscope and with the krypton/argon laser (488 nm and 568 nm) equipped for excitation. The FITC image was taken under a 522/35 filter set, while the TRITC image was obtained using a 580/32 filter set. Serial optical sections were observed and stored on computer for each specimen. The images of microtubules and actin filaments were processed on computer using two image-processing programs (Bio-Rad confocal assistant and Adobe Photoshop, version 3.0, Adobe System Inc.). To measure the fluorescence intensity of the microtubules, the fluorescence and the area of the gill were measured using the Bio-Rad COMOS program.

Effects of WGA and colchicine on the fluorescence intensity of microtubules in the gills

Three groups of five larvae were selected from the same brood of tilapia 5 days post-hatching. One group was kept in local fresh water. Another group was treated with WGA (40 ng ml^{-1}) in local fresh water for 30 min. The third group was treated with colchicine (2×10^{-4} mol l^{-1}) in local fresh water for 4 h. All treatments were at 27 °C. After incubation,

Table 1. *Composition of Taipei local fresh water and three artificial hypotonic media*

	$[Ca^{2+}]$ (mmol l^{-1})	$[Mg^{2+}]$ (mmol l^{-1})	$[Na^+]$ (mmol l^{-1})	$[K^+]$ (mmol l^{-1})
Fresh water	0.201±0.009	0.157±0.003	0.420±0.067	0.027±0.003
$[Ca^{2+}]$ 0.2	0.232±0.062	0.155±0.012	0.654±0.053	0.402±0.018
$[Ca^{2+}]$ 0.02	0.015±0.005	0.140±0.005	0.578±0.057	0.345±0.054
$[Ca^{2+}]$ 0.002	0.002±0.001	0.142±0.005	0.580±0.037	0.345±0.024

Values are means ± S.E.M. (N=5).

the gills were excised, stained for α-tubulin as described above, and the fluorescence intensity of the microtubules was determined.

Effects of WGA treatment on Ca^{2+} levels

An electrode for measuring Ca^{2+} levels (Cole-Parmer Instrument Company, USA) showed that the Ca^{2+} concentration did not change after the addition of WGA or colchicine to the water. This confirmed that WGA does not bind free Ca^{2+} in the water. Ca^{2+} influx rate was measured as described by Hwang *et al.* (1994, 1996). Fifteen containers were prepared and divided equally into three sets. Three media differing in Ca^{2+} concentration (0.2, 0.02 or 0.002 mmol l^{-1}, see Table 1) were placed into each set of five 100 ml containers. $^{45}CaCl_2$ (32.9 Bq mmol^{-1}, Amersham, UK) was used as the tracer and added to each container at 0.015 Bq mmol^{-1}. In each set, one container acted as a control (no WGA). To the other four containers was added 10, 20, 30 or 40 ng ml^{-1} WGA. Fifteen groups of 10 larvae were selected from the same brood of tilapia 5 days post-hatching. The larvae were rinsed briefly three times in distilled water to remove any Ca^{2+} adhering to the body surface and then transferred to the 15 containers and incubated for 30 min at 27 °C. Five fish were then used for the determination of Ca^{2+} influx rate. The fish were removed and washed three times for 1 min each in non-radioactive ice-cold fresh water to remove any isotope on the body surface and also to anaesthetize the larvae. They were then treated with tissue solubilizer (Soluene 350, Packard, USA) at 60 °C for 6 h. The digested preparations were mixed with scintillation fluid (Hionic-Fluor, Packard, USA), and the radioactivity was counted in a liquid scintillation β-counter (1211 Rackbeta, LKB, Finland). Ca^{2+} influx rate was determined using the formula:

$$J_{\mathrm{in}} = \frac{Q_{\mathrm{larva}}}{X_{\mathrm{out}}TW},$$

where J_{in} is influx rate (pmol mg^{-1} h^{-1}), Q_{larva} is the radioactivity of the larva (cts min^{-1} individual^{-1}) at the end of the incubation, X_{out} is the specific activity of the incubation medium (cts min^{-1} pmol^{-1}), T is incubation time (30 min) and W is wet body mass (mg). Preliminary experiments confirmed that the use of $^{45}Ca^{2+}$ did not affect the development of the larvae. The different quenching effects of water and tissue were taken into account before the calculations were performed, as described previously (Hwang *et al.* 1994). The wet body mass was determined from a different group of 10 larvae, which were rinsed briefly in double-deionized water, wiped with filter paper to remove excess solution on the body surface, and weighed on an electronic balance (Mettler Toledo, Switzerland) to 0.1 mg.

Effects of colchicine treatment on Ca^{2+} influx rate

Tilapia larvae from a single brood were subdivided into five groups, 15 individuals in each group. One group of larvae was reserved as controls. The other four groups of larvae were treated with 0.05, 0.1, 0.015 or 0.2 mmol l^{-1} colchicine in local fresh water for 4 h at 27 °C. The larvae then were processed to determine Ca^{2+} influx rate as described above.

Scanning electron microscopy

Gills were removed from larvae 5 days post-hatching, fixed in phosphate-buffered 4 % paraformaldehyde plus 2 % glutaraldehyde, pH 7.4, for 8 h at 4 °C, and then in phosphate-buffered 1 % osmium tetroxide, pH 7.4, for 1 h at 4 °C. The tissues were dehydrated in ascending concentrations of ethanol from 30 % to 100 %, in 100 % acetone, and then dried using a Hitachi HCP-2 critical-point drier. After sputter-coating for 3 min with a gold–palladium complex using an Eiko 1B-2 vacuum evaporator, the specimens were examined using a scanning electron microscope (Hitachi S-2500).

Statistics

Values were compared using a one-way analysis of variance (ANOVA) (Tukey's pair-wise method). Values are presented as means ± S.E.M. unless stated otherwise.

Results

Microtubule and actin filaments in gill epithelial cells

Staining for α-tubulin revealed that microtubules are not evenly distributed in the gills of tilapia larvae. Microtubules are seen as threads in certain locations in the gill epithelium only (Fig. 1A). Double staining for α-tubulin and Na^+/K^+-ATPase showed that most of the filamental networks exactly matched regions of high Na^+/K^+-ATPase density (Fig. 1B). The apical openings of the MR cells were especially abundant in microtubules when compared with other regions of the gill. Cross section of the gills (Fig. 1C) showed that they are arranged in bundles centred on the apical openings of the MR cells and expand towards the base of the cells. The actin filaments, stained with phalloidin–TRITC, in contrast, are mainly distributed in the surface cells of the gill epithelia (Fig. 2A). The MR cells and the lower layer of epithelial cells are weakly stained, as shown by double-staining for Na^+/K^+-ATPase (Fig. 2B) and in the cross sections of gills (Fig. 2C). A comparison of the double-stained feature (Fig. 2B) with the scanning electron micrograph (Fig. 2D) shows that actin staining provides a good match to the microridges in the surface cells of the gill epithelia and to junctions between the epithelial cells and MR cells.

Effects of WGA and colchicine on the fluorescence intensity of microtubules in the gills

Treatment with 40 ng ml^{-1} WGA resulted in an increase in the fluorescence intensity of the microtubules in the gills. In the gills of control larvae (Fig. 3A), bundles of microtubules are located in the MR cells. The fluorescence intensity in control gill was 47.5±12.8 pixels mm^{-2} gill (Fig. 4). In the larvae treated with 40 ng ml^{-1} WGA (Fig. 3B), microtubule staining was more intense. Bundles of microtubules were much

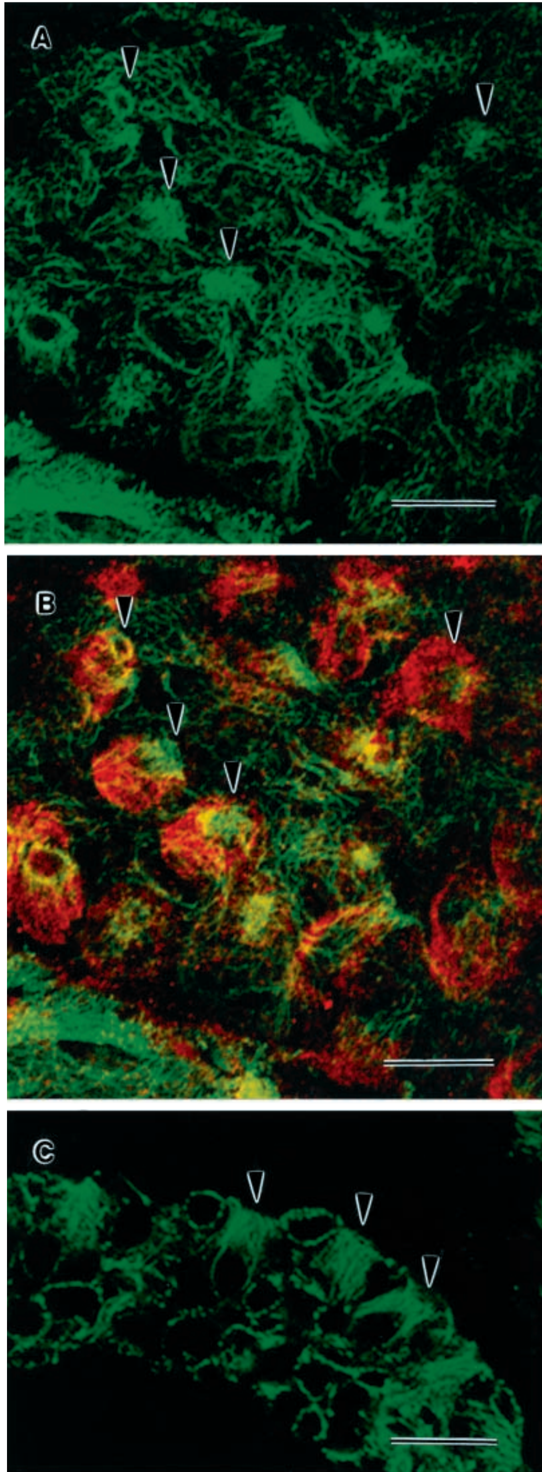

Fig. 1. (A) FITC staining for α-tubulin in the gills of a tilapia larva exposed to control fresh water. (B) Double-staining for α-tubulin using FITC and for Na^+/K^+-ATPase using TRITC in the same area of the gill as in A. Microtubules appear as green threads at the apical regions of the mitochondria-rich (MR) cells in which Na^+/K^+-ATPase is labelled red/yellow. (C) A cross section of gill stained for α-tubulin. The microtubules are arranged in bundles, centred in the apical openings of the MR cells, and expand towards the base of the MR cells. Scale bars, 10 μm. Arrowheads indicate the apical openings of the MR cells.

more conspicuous than in the control gills, and the fluorescence intensity was 85.7±22.6 pixels mm^{-2} gill (Fig. 4). In contrast, bundles of microtubules were no longer apparent after treatment with 0.2 mmol l^{-1} colchicine (Fig. 3C). The fluorescence intensity was significantly lower than that of both other groups at 21.7±8.9 pixels mm^{-2} gill (Fig. 4). The staining pattern of the actin filaments was not changed by WGA treatment (data not shown).

Effects of WGA treatment on Ca^{2+} uptake

Fig. 5A gives Ca^{2+} influx rates for larvae exposed to 0.2 mmol l^{-1} Ca^{2+} and different WGA concentrations. Ca^{2+} influx rate differed from the control value only for fish exposed to 40 ng ml^{-1} WGA. A similar result was obtained for larvae exposed to 0.02 mmol l^{-1} Ca^{2+} (Fig. 5B). However, in fish exposed to 0.002 mmol l^{-1} Ca^{2+} (Fig. 5C), Ca^{2+} influx rates differed significantly from control values for WGA concentrations of 20, 30 and 40 ng ml^{-1}, these values being more than 50 % higher than the control value. Therefore, WGA enhanced Ca^{2+} influx rates of tilapia larvae compared with the control larvae, particularly when the larvae were kept in a medium in which [Ca^{2+}] was extremely low. It should be noted here that Ca^{2+} influx rates are known to decrease as environmental levels of Ca^{2+} decline (Hwang *et al.* 1996; compare Fig. 5A–C).

Effects of colchicine treatment on Ca^{2+} influx rate

Fig. 6 shows the Ca^{2+} influx rates of control and colchicine-treated larvae. Treatment with 0.2 mmol l^{-1} colchicine cause a significant reduction in Ca^{2+} influx in comparison with the control group and the groups treated with lower doses of colchicine.

Discussion

A previous study suggested that the WGA binding sites in the apex of MR cells are likely to be involved in the uptake of Ca^{2+} because the number of these binding sites increases in the gills of tilapia adapted to low-[Ca^{2+}] water (Tsai and Hwang, 1998). The present study demonstrates that Ca^{2+} influx rates of tilapia larvae are indeed raised by adding WGA to the ambient water, particularly in a low-[Ca^{2+}] medium. WGA may provide ligands that interact with the WGA binding sites in the gills and thus induce cellular activity that facilitates Ca^{2+} absorption. This study provides direct evidence that the WGA binding sites at the apex of MR cells function in Ca^{2+} uptake and may be activated in response to a low-[Ca^{2+}] environment.

The present study also reveals that microtubules are the major cytoskeletal element in the MR cells of tilapia gills. Treatment with WGA increased the fluorescence intensity of the microtubules in gills and concurrently enhanced Ca^{2+} uptake rates. Treatment with colchicine, in contrast, reduced both the fluorescence intensity of the microtubules and the Ca^{2+} influx rate. Together, these two results confirm that the microtubules play a critical role in Ca^{2+} uptake in tilapia gills. Synthesis or decomposition of the microtubules in tilapia gill

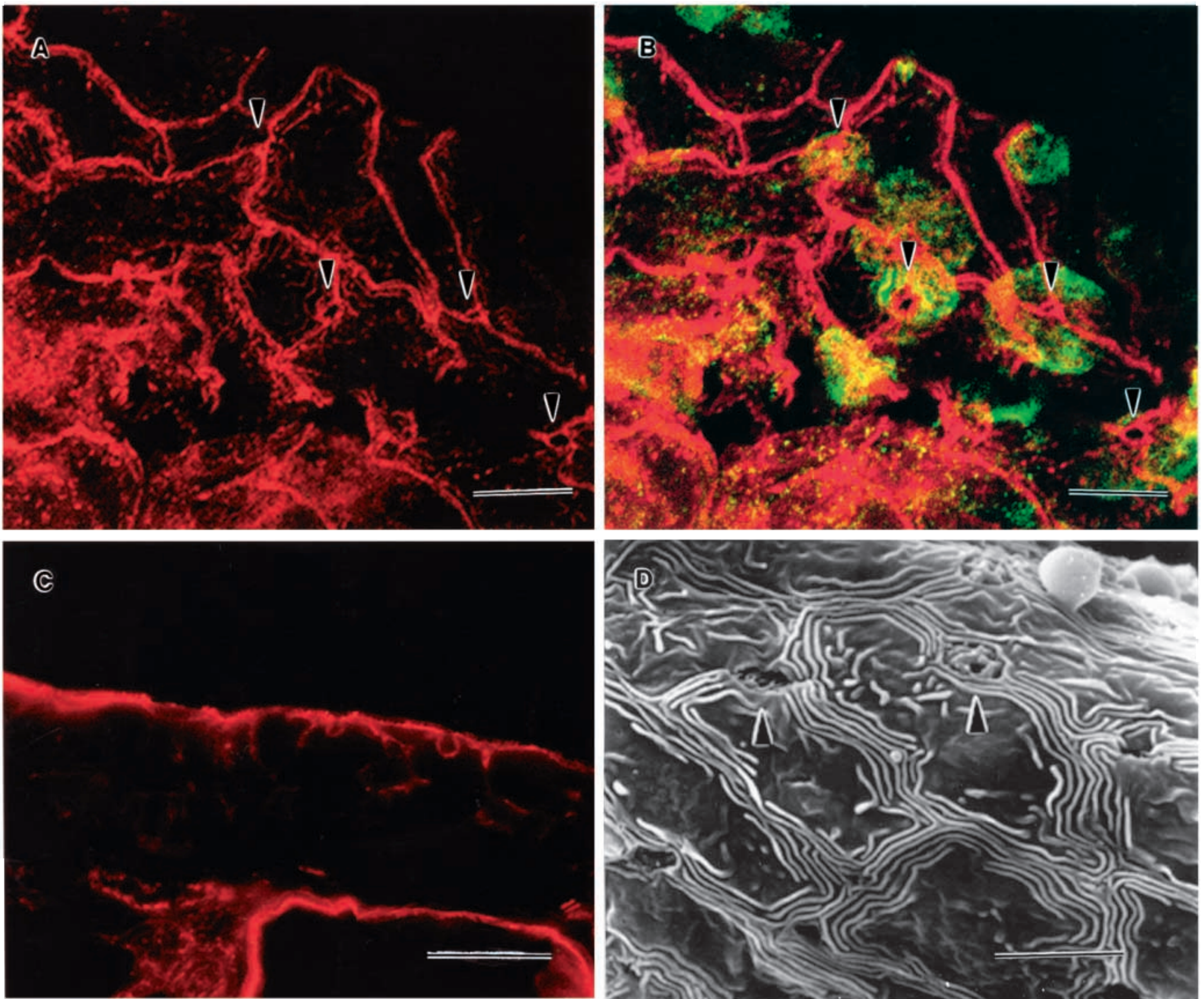

Fig. 2. (A) Phalloidin-conjugated TRITC staining of actin filaments at the surface of the gill of a tilapia larva. (B) Double-labelling of actin filaments (stained using TRITC) and Na^{+}/K^{+}-ATPase (stained using FITC) in the gills of a tilapia larva. The actin filaments (red) are mainly distributed in the surface epithelial cells and are scarce in the mitochondria-rich (MR) cells, which are identified by Na^{+}/K^{+}-ATPase staining (green/yellow). The actin filament image was taken from the gill surface. The Na^{+}/K^{+}-ATPase image was taken 3 µm below the gill surface. (C) A cross section of gill stained for actin using phalloidin–TRITC. (D) A scanning electron micrograph of the gill surface. Arrowheads indicate the apical openings of the MR cells. Scale bars, 10 µm.

following WGA or colchicine treatment, as indicated by the observed change in fluorescence intensity, will affect the cells' ability to take up Ca^{2+} from the environment. In addition, because the WGA binding sites and the microtubules are both located at the apex of the MR cells, it is possible that these two components are linked in the processes accompanying Ca^{2+} uptake. In contrast, actin filaments were scarce in the MR cells and were mainly distributed at the junctions and microridges of the surface cells of the gill epithelia. No changes were found in the staining pattern of the actin filaments in gills following WGA treatment. Therefore, the actin filaments are unlikely to be involved in the activity of the WGA binding sites. Nevertheless, our data cannot exclude the possibility that the actin filaments are involved in other processes connected with ionic regulation in tilapia gills.

Maetz and Pic (1977) proposed a possible link between microtubules and the bulk transport of NaCl through vesicles across the apex of the MR cells. When the gills of seawater-adapted mullet (*Mugil capito*) were treated with colchicine, there was a net influx of Na^{+} and Cl^{-}. While our data showed that Ca^{2+} influx rate was reduced by colchicine treatment in freshwater-adapted tilapia, it is well known that fish gills

actively excrete NaCl in salt water and take up NaCl in fresh water. Therefore, these results suggest that disruption of the microtubules by colchicine blocks the normal processes in both saltwater and freshwater fish.

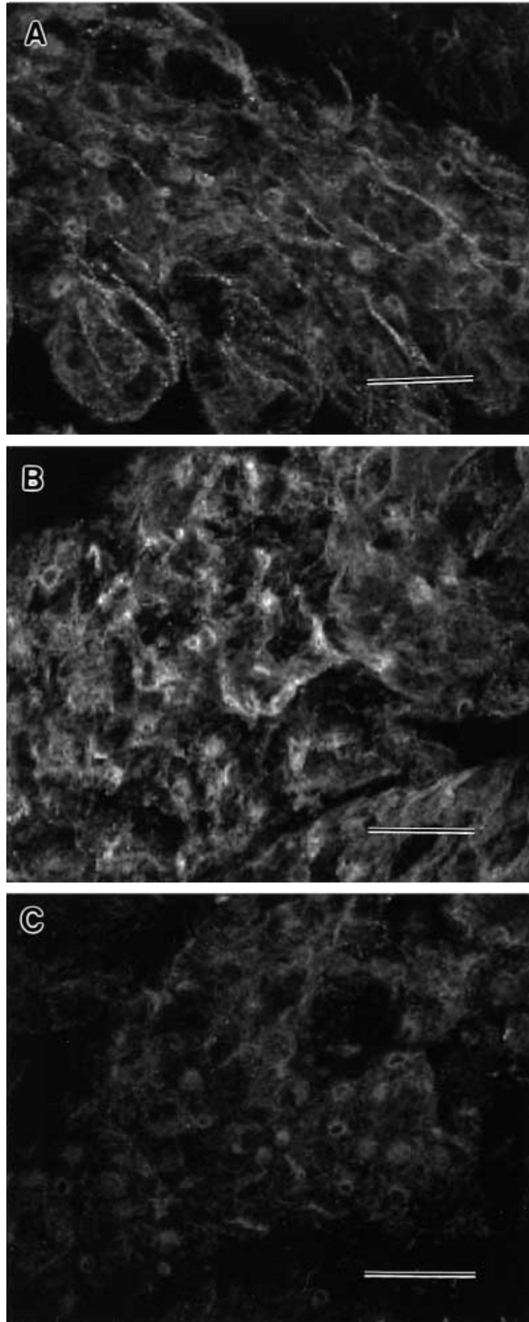

Fig. 3. Fluorescence of microtubules stained using FITC in the gills of a tilapia larva treated with wheat germ agglutinin (WGA) and colchicine. These images are surface views of the gill epithelium. (A) Control (no WGA or colchicine). (B) Larva treated with 40 ng ml^{-1} WGA for 30 min. The bundles of microtubules are much more conspicuous and the intensity of microtubule fluorescence has increased. (C) Larva treated with 0.2 mmol l^{-1} colchicine for 4 h. The bundles of microtubules are no longer visible and the fluorescence intensity is reduced. Scale bars, 50 μm.

Flik and Verbost (1995) proposed a model describing the cellular mechanisms for the transport of Ca^{2+} in branchial epithelium with special reference to the chloride (MR) cells. They suggested that absorption of Ca^{2+} in the apical membrane is mediated by specific channels or carrier molecules. The Ca^{2+}-ATPase in the basolateral membrane would provide the major driving force for extruding Ca^{2+} into the blood. In the studies investigating Ca^{2+} regulation in the MR cells of fish gills, however, the structure and organization of the cellular components have been largely ignored. Mays *et al.* (1995) found that the architecture of cytoskeletal elements must be correct to establish cell polarity and for certain cellular events to be completed. In epithelial cells, the apical and basolateral plasma membranes generally differed in structure, and molecules such as channels, transporters, receptors and enzymes also differ in these two regions; this allows modulation of cellular physiology for the transport of ions, fluid and large molecules across the epithelia (Fath *et al.* 1993; Bradbury and Bridges, 1994; Hensen *et al.* 1995; Verrey *et al.* 1995; Brown and Stow, 1996). In such studies, the microtubules have frequently been characterized for the transport of Ca^{2+}. In the proximal tubule cells of the mammalian kidney, for example, a change in apical membrane structure and a decrease in the number of vesicles in apical regions were induced by colchicine treatment, and these effects were suggested to be correlated with the redistribution of aquaporin gp330 for Ca^{2+} binding (Christensen *et al.*1992; Elkjaer *et al.* 1995). In the chick intestine, redistribution of calbindin-D_{28k}, a protein with affinity for Ca^{2+} and functioning in Ca^{2+} absorption in intestinal cells, was similarly found to accompany the reorganization of microtubules following colchicine treatment (Nemere *et al.* 1991).

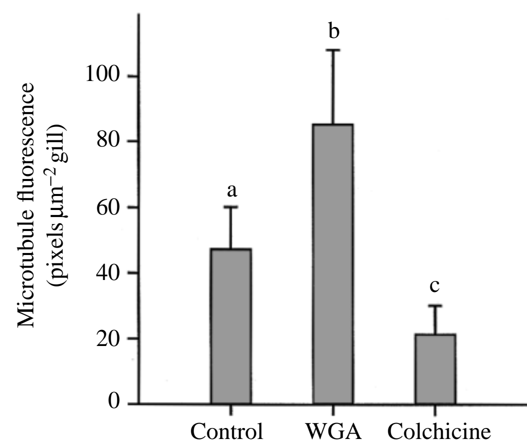

Fig. 4. Comparison of the intensity of fluorescence of microtubules stained with FITC in gills of tilapia larvae treated with 40 ng ml^{-1} wheat germ agglutinin (WGA), with 0.2 mmol l^{-1} colchicine or untreated (control). Values are means + S.E.M. (*N*=5). Fluorescence intensity was determined on computer as pixel intensity. Different letters indicate significantly different values (one-way ANOVA, Tukey's pairwise comparisons).

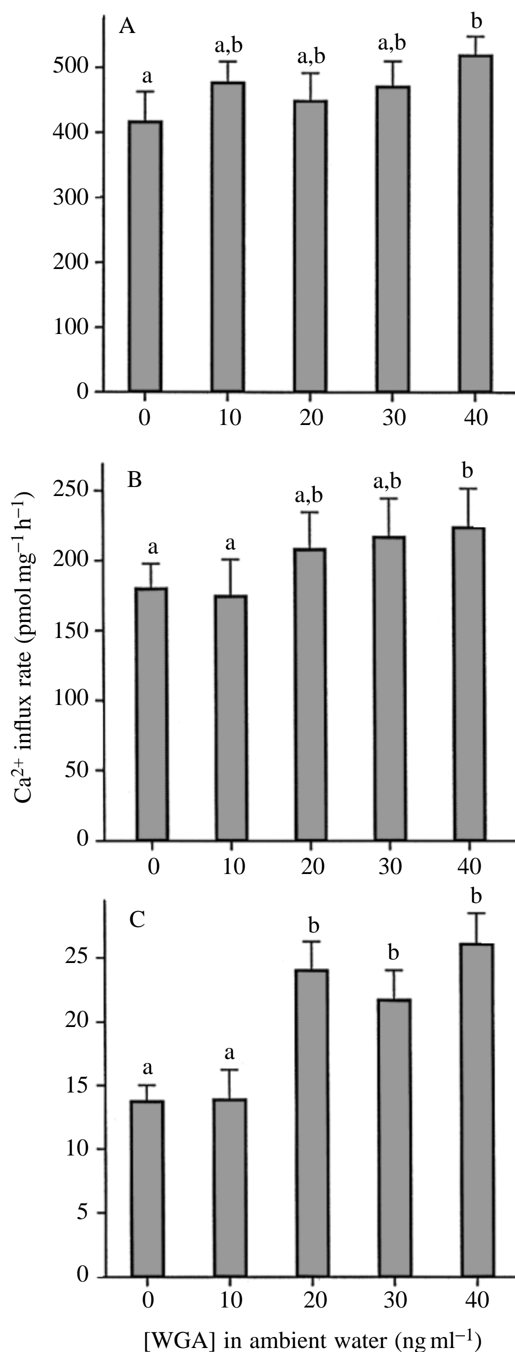

Fig. 5. Effects of exposure to wheat germ agglutinin (WGA) on Ca^{2+} influx rate in tilapia larvae exposed for 30 min to three artificial media which differed in [Ca^{2+}]. (A) [Ca^{2+}]=0.2 mmol l^{-1}; (B) [Ca^{2+}]=0.02 mol l^{-1}; (C) [Ca^{2+}]=0.002 mmol l^{-1}. Values are means + S.E.M. (N=8–10). Different letters indicate significantly different values (one-way ANOVA, Tukey's pairwise comparisons).

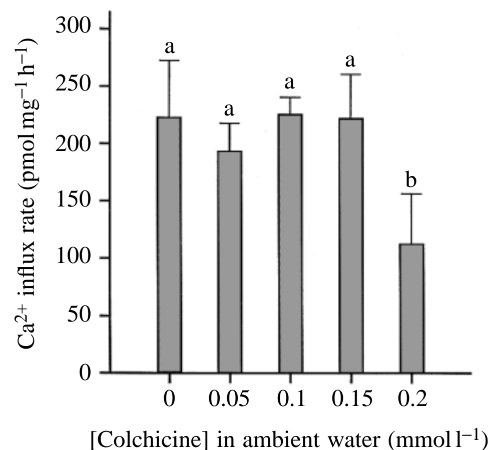

Fig. 6. Effect of colchicine treatment on Ca^{2+} influx rate of tilapia larvae exposed to [Ca^{2+}]=0.2 mmol l^{-1}. Values are means + S.E.M. (N=8–10). Different letters indicate significantly different values (one-way ANOVA, Tukey's pairwise comparisons).

The role of the microtubules at the apex of MR cells and their participation in the cellular mechanisms of Ca^{2+} uptake should be investigated. These microtubules may be critical in establishing apical and basolateral polarity in the MR cells. The functions of channels or carriers involved in Ca^{2+} absorption in the MR cells are dependent on the correct arrangement of the microtubules.

The characterization of microtubule networks in MR cells and the effects of WGA and colchicine on the MR cells will, in turn, provide insights into results from recent studies regarding the MR cells. These studies have indicated that the morphology of the apical openings in MR cells is flexible in responding to various conditions (Avella *et al.* 1987; Laurent and Perry, 1990; Perry *et al.* 1992; Pisam *et al.* 1995; Lee *et al.* 1996*a*,*b*). In freshwater trout (*Oncorhynchus mykiss*) gills, the apical surface area of the MR cells increases significantly after cortisol injection or after transfer of the fish into ion-deficient water. Moreover, the increase in apical surface area of the MR cells was demonstrated to be related to ion uptake (Laurent and Perry, 1990). In the gills of Atlantic salmon (*Salmo salar*) and tilapia (*Oreochromis niloticus*), two types of MR cells, α and β cells, were characterized using transmission electron microscopy (Pisam *et al.* 1995). Differences in the apical membrane between these two types of cell were identified. The surface membranes of the α cells are much more elongated than those of the β cells. Not surprisingly, the α and β cells are suggested to differ in ionic uptake and are regulated differently by growth hormone and prolactin. In our laboratory, three types of apical openings of the MR cells were described by using scanning electron microscopy in freshwater-adapted tilapia (*Oreochromis mossambicus*) and carp (*Cyprinus carpio*). These three types were termed wavy convex, shallow basin and deep hole MR cells (Lee *et al.* 1996*b*) and varied in the width of the apical opening and in the number of microvilli within it. Each type of MR cell was found to be dominant in a medium of a particular ionic concentrations. Moreover, the morphological changes in MR cells were found to be reversible and occurred within a couple of hours following transfer into a new medium. Maetz and Pic (1977), using transmission electron microscopy,

observed that the microvilli in the apical openings of the MR cells degenerated after colchicine treatment. This implies that microtubules may be fundamental to the structure of microvilli. The morphological modifications in the MR cells described previously could also be due to the reorganization of microtubules. Moreover, the relationship between structural changes in the apex of MR cells and the absorption of ions proposed previously is confirmed by the present results, in which the rate of Ca^{2+} influx is reduced after disruption of the microtubules by colchicine.

The dosage of WGA used in the present study was quite low. In studies examining the effects of WGA on cytoskeletal structure (Sjolander and Magnusson, 1988; Danilov and Cohen, 1989; Arena *et al.* 1990; Lin and Huestis, 1995), actin filaments were affected by the addition of 1–10 $\mu g\,ml^{-1}$ WGA to the medium. In the epithelial cells of the frog urinary bladder, up to 100 $\mu g\,ml^{-1}$ WGA was needed to induce profound cell surface changes and concurrently to reduce movement of water across the epithelium (Favard *et al.* 1989). However, only 40 $ng\,ml^{-1}$ WGA in the ambient water, a 25–2500-fold lower dose than those used in previous studies, was sufficient to modulate the microtubule network in the apex of MR cells and the rate of Ca^{2+} influx in the gills of tilapia larvae. It may be that the microtubule network in the MR cells of tilapia gills is much more sensitive to WGA or that the WGA binding site has a more pivotal role in adjusting the microtubule network in these MR cells.

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