Ca$^{2+}$ and Mg$^{2+}$ Transport in Gills and Gut of Tilapia, Oreochromis mossambicus: A Review

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ABSTRACT

The euryhaline tilapia Oreochromis mossambicus kept in fresh water takes up calcium mainly from the water via the gills, like other freshwater fish. In the gills specialized mitochondria-rich cells, the chloride cells, are thought to mediate a transcellular Ca$^{2+}$ transport. Second messenger operated calcium channels (SMOCs) in the apical membrane, regulated by the hormone stanniocalcin, allow minute-to-minute control over the entry of Ca$^{2+}$. In the basolateral plasma membranes of these cells, an ATP-consuming Ca$^{2+}$ transporting enzyme provides the major driving force for extrusion of Ca$^{2+}$ into the blood; in addition, an Na$^+$/Ca$^{2+}$ exchanger is present in these membranes. The kinetics of the exchanger in vitro indicate that this extrusion mechanism dominates when intracellular calcium levels reach micromolar levels. In the gills, the transport of calcium appears primarily ATPase mediated and therefore largely independent of the Na$^+$ status of the transporting cell. In enterocytes, similar mechanisms for transcellular transport of Ca$^{2+}$ exist. However, in the intestinal epithelium the extrusion of Ca$^{2+}$ is primarily via the Na$^+$/Ca$^{2+}$ exchanger and to a very limited extent mediated via the Ca$^{2+}$-ATPase. Indeed, calcium transport over the intestinal epithelium is dependent on the Na$^+$ status and the Na$^+$/K$^+$-ATPase activity of the epithelium. Prolactin and cortisol are endocrine factors determining the relative densities of calcium pumps in basolateral plasma membranes.

At least 80% of the magnesium required for growth and homeostasis is absorbed from the food via the intestine. Magnesium is transported transcellularly and actively via enterocytes. The movement of Mg$^{2+}$ over the apical membrane is passive, down an electrochemical gradient. The cytosolic Mg$^{2+}$ concentration is kept well below its equilibrium concentration. The extrusion over the basolateral plasma membrane is mediated by an ATP-consuming enzyme. The gills contribute less than 20% to magnesium uptake, but up to 50% in tilapia fed a magnesium deficient diet. Evidence is accruing that prolactin is involved in the adaptation to low magnesium diets. © 1993 Wiley-Liss, Inc.

Our laboratories have conducted studies pertaining to calcium and magnesium transport mechanisms in fish gills and intestinal epithelium. This is a review of published studies and recent unpublished findings resulting from this work. It is not an exhaustive review; rather, data sets on tilapia research (some still incomplete) are presented to stimulate discussion.

CALCIUM TRANSPORT

Gills

In the euryhaline teleost tilapia Oreochromis mossambicus kept in fresh water, the gills are the major site for exchange of calcium with the water (Flik et al., '86a,b). Modulation of branchial calcium fluxes in response to particular environmental water conditions (e.g., variations in calcium concentrations) is therefore a prerequisite for calcium homeostasis. Consequently, the dominant calcitropic hormones in fish (stanniocalcin, prolactin, and cortisol; see below) exert major effects on the gills.

Only recently have the mechanisms underlying the unidirectional fluxes of calcium through the branchial epithelium been elucidated. Calcium taken up from the water in freshwater fish follows a transcellular, hormone-controlled pathway located in the chloride cells of the gills. In a model for transcellular Ca$^{2+}$ transport (Fig. 1) based on our studies on trout, eel, and tilapia, stanniocalcin (STC)
controls the apical membrane transition of Ca\(^{2+}\) (Lafeber et al., '88; Flik, '90), while cortisol (Flik and Perry, '89) and prolactin (Flik et al., '84b, '86c, '89a) influence the enzymic makeup of the basolateral plasma membrane. The latter two hormones increase the Ca\(^{2+}\)-ATPase density, and by doing so determine the calcium pumping capacity of the chloride cells and hence of the branchial epithelium. STC is a fast-acting hormone inhibiting calcium influx within minutes (Lafeber et al., '88). Cortisol and prolactin effects on the epithelium become clear only after several days, suggesting that new cells have developed stimulated by a particular hormonal status of the animal. This status in turn represents the adaptive response of the fish to the changed environment.

**Ca\(^{2+}\) entry over the apical membrane**

STC is a homodimeric glycoprotein of 56 (in most fish studied) to 60 (in eel) kilodalton from the corpuscles of Stannius (Flik et al., '89b, '90a). Physiological studies have provided circumstantial evidence that STC is the major hypocalcemic hormone in fish exerting the minute-to-minute control over apical membrane Ca\(^{2+}\) transport. In a trout, isolated head preparation STC inhibits Ca\(^{2+}\) influx within 15 min (Lafeber et al., '88). STC also inhibits Ca\(^{2+}\) uptake from the water into branchial epithelium (Verbost et al., '89). These observations are in line with a conclusion reached earlier, down an electrochemical gradient through voltage-independent calcium channels (Perry and Flik, '88). Removal of the corpuscles of Stannius (rapidly) evokes a patent hypercalcemia (Hanssen et al., '89) and the severity of the hypercalcemia is positively correlated with the water calcium level (Flik, '90). These observations all indicate that Ca\(^{2+}\) from the water enters the Ca\(^{2+}\) transporting cell via an STC-controlled pathway located in the apical membrane.

We postulate that the receptors for STC are localized in the basolateral plasma membrane. Subsequently, second and third messengers transduce the STC signal to the apical membrane; this is the reasoning behind the postulate of second messenger operated calcium channels (SMOCs). An extract of trout corpuscles of Stannius lowers the branchial cAMP content and inhibits adenylyl cyclase in branchial (and in renal and intestinal) plasma membranes (Flik, '90). These results suggested that cAMP is involved as a second messenger in the STC signal transduction. However, recent studies have revealed that an extract of corpuscles of Stannius contains two bioactive components, STC and a small glycoprotein which we tentatively equate with teleocalcin, first reported on by Ma and Copp ('78). It turned out that the "cAMP-effects" in an extract of the corpuscles of Stannius must be attributed to the teleocalcin activity in the extract. This effect on cAMP levels, however, is unrelated to the direct regulation of the calcium influx, as pure teleocalcin has no effect on calcium influx (P.M. Verbost, personal communication). It is our strong belief that inositol metabolites are involved in the messenger pathway of STC. The impetus for this statement was the observation that STC stimulated the production of sn-1,2-diacylglycerol (DAG) in tilapia branchial cells (Fig. 2). DAG is one of the second messengers released in a cell when the inositol cycle is stimulated. Studies on the effects of STC on the production of inositol metabolites are now being carried out. One would predict that IP\(_3\), the other second messenger produced when inositol metabolism is stimulated, is produced in the branchial cells after stimulation with STC. It is usually found in this field of research that IP\(_3\) levels are low and difficult to establish. In line with our prediction is the observation that in permeabilized gill cells IP\(_3\) stimu-
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Fig. 2. Stanniocalcin (STC) stimulation of sn-1,2-diacylglycerol (DAG) production in freshwater tilapia gill cells. DAG was determined with a commercial radioenzymatic assay kit (RPN200, Amersham). Mean values ± SEM for six fish are given. The asterisk indicates significance of the difference compared to the control (*P < 0.03; Mann-Whitney U-test).

ulates Ca\(^{2+}\) (third messenger) release from endoplasmic reticular stores (Fig. 3). Thus, although IP\(_3\) production per se as a response to an STC stimulus still has to be established, the presence of an IP\(_3\) receptor in the endoplasmic reticulum of gill cells seems evident.

The overall picture emerging so far is that STC evokes in its targets second and third messenger responses, which are tentatively related to the control of the apical membrane Ca\(^{2+}\) channel: the time course of the STC inhibition of the branchial Ca\(^{2+}\) influx (significant effects within 15 min) is in accordance with the rapid second messenger responses observed (effects within 10 min). Phosphorylation studies with highly purified apical, i.e., brush border, preparations of intestinal and renal tissue may ultimately give more direct evidence for Ca\(^{2+}\) mediated modulatory actions of STC on SMOCs in these cells. Branchial apical membranes are difficult to isolate, however.

**Ca\(^{2+}\) extrusion in the basolateral plasma membrane**

Two mechanisms for Ca\(^{2+}\) extrusion exist in the basolateral plasma membrane of branchial epithelium. The first, a high-affinity Ca\(^{2+}\)-ATPase, represents the ATP-driven calcium pump and is well documented (Flik et al., '84a, '85a,b; Perry and Flik, '88). The second is an Na\(^+\)/Ca\(^{2+}\) exchange mechanism (Figs. 4 and 5a). We present here for the first time evidence for its presence in branchial epithelial plasma membranes.

ATP-driven Ca\(^{2+}\) transport and Na\(^+\)/Ca\(^{2+}\) exchange activity were analyzed in the same plasma membrane preparation. The methodology for determining Ca\(^{2+}\)-ATPase mediated Ca\(^{2+}\) transport in plasma membrane vesicles (determined as the difference in \(^{45}\)Ca\(^{2+}\) accumulation in the presence and absence of ATP) has been described extensively in the references given; that for Na\(^+\)/Ca\(^{2+}\) exchange will be briefly described below. The kinetics of the Ca\(^{2+}\) pump (for tilapia we found a \(K_{0.5}\) of 102 ± 46 nM Ca\(^{2+}\) and a \(V_{\text{max}}\) of 3.79 nmol \(\times\) min\(^{-1}\) per mg protein; \(n = 6\)) suggest that this enzyme operates in the extrusion process (Flik et al., '85a). Typically, the enzyme is stimulated in vitro at calcium levels that are found at rest in most cells (around 100 nM) and the maximum Ca\(^{2+}\) transport capacity of the gills derived from the \(V_{\text{max}}\) (Flik et al., '85a) is realistic when compared to the Ca\(^{2+}\) influx that is observed in the gills. In addition to thermodynamic arguments (Perry and Flik, '88), further evidence for a role of the ATP-driven calcium pump in transepithelial transport comes from endocrinological studies. Hypercalcemic hormones, viz. cortisol in trout (Flik and Perry, '89) and prolactin in eel (Flik et al., '84b, '89a) and tilapia (Flik et al., '86c), stimulate Ca\(^{2+}\) influx via the gills and increase the density of the calcium pumps in the basolateral plasma membranes.

Characteristic changes in branchial epithelial plasma membranes observed by us after treatment of the fish with prolactin or cortisol are a higher activity of Ca\(^{2+}\)-ATPase relative to Na\(^+\)/K\(^+\)-ATPase and an increased \(V_{\text{max}}\) of the calcium pumps in basolateral plasma membrane vesicles. Based on the rapid turnover of chloride cells in the gills (Wendelaar Bonga et al., '90), it is our strong belief that cortisol and prolactin administration for 4–8 days (the time period required to evoke hypercalcemic responses) results in the development of a new population of chloride cells that reflect the particular hormonal status.

The first indication for Na\(^+\)/Ca\(^{2+}\) exchange activity in branchial epithelial plasma membranes came from an experiment in which Ca\(^{2+}\) transporting vesicles (ATP-dependent transport at 1 \(\mu\)M Ca\(^{2+}\) in the medium) were challenged after 10 min of calcium loading with 70 mM NaCl or KCl (control). The inwardly directed Na\(^+\) gradient thus created drives out \(^{45}\)Ca\(^{2+}\) accumulated in the vesicles as a result of the ATPase activity. The loss of \(^{45}\)Ca\(^{2+}\) seen when KCl is added to the medium is assumed to result from an osmotic effect. Significantly greater loss of \(^{45}\)Ca\(^{2+}\) occurred at 1 and 5 min after addition of NaCl compared to KCl (Fig. 4).
CALCIUM AND MAGNESIUM TRANSPORT IN TILAPIA

Fig. 3. IP3 induced Ca²⁺ release in freshwater tilapia permeabilized gill cells. Isolated gill cells were permeabilized with saponin. ⁴⁶Ca²⁺ uptake was assayed in a Leibowitz medium supplemented with 3 mM ATP, 667 MBq/ml ⁴⁶CaCl₂ (Amersham), and a calculated 0.1 μM Ca²⁺, using an EGTA/NTA/HEEDTA buffer system (Flik et al., '90b). After the ⁴⁶Ca²⁺ uptake had reached a plateau, inositol (1,4,5) trisphosphate (Calbiochem) was added to a final concentration of 10 μM. Control experiments included the omission of ATP and the addition of oxalate and vanadate in the presence of ATP (data not shown). The addition of IP₃ (arrow) results in the release of Ca²⁺ out of a compartment that is oxalate-permeable and equipped with a vanadate-inhibitable Ca²⁺ pump with high affinity for Ca²⁺ (the endoplasmic reticulum). Values ± SEM are given for 6 fish. Release of Ca²⁺ occurred in all cases.

We then further investigated the calcium kinetics of this presumed exchange activity as follows. Na⁺-dependent Ca²⁺ transport across plasma membranes was assayed as the difference in ⁴⁶Ca²⁺ accumulation upon transfer of membrane vesicles equilibrated in 150 mM NaCl to media containing either 150 mM NaCl (blank) or 150 mM KCl. In composing the media, a 25-fold dilution of the vesicle suspension was taken into account to yield the following final concentrations: 150 mM NaCl or KCl, 20 mM HEPES/Tris (pH 7.4), 0.5 mM EGTA, 0.5 mM HEEDTA, 0.5 mM NTA, 0.8 mM free Mg²⁺, and 7.5 × 10⁻⁵ to 5 × 10⁻² mM free Ca²⁺. Free Ca²⁺ and Mg²⁺ concentrations were calculated as described in detail recently (Schoenmakers et al., '92b). The ⁴⁶Ca radioactive concentration was 0.5 to 0.8 MBq × ml⁻¹. A 5 μl vesicle suspension was mixed with 120 μl medium, both prewarmed to 37°C. After 5 sec of incubation, the reaction was quenched by addition of 1 ml ice-cold stop buffer (150 mM NaCl, 20 mM HEPES/Tris at pH 7.4, 1.0 mM LaCl₃) to the incubate. Membrane vesicles with retained ⁴⁶Ca were collected by filtration over 0.45 μm filters (Schleicher & Schull, ME 25); the filters were washed twice with 2 ml stop buffer. The filters were dissolved in the scintillation fluid (Aqualuma⁷, Lumac) and the radioactivity collected on the filters was determined by liquid scintillation counting. We first analyzed the Ca²⁺ dependence of the exchanger to allow an evaluation of the relative activities of the ATPase and the exchanger in the membrane as a function of the (cytosolic) calcium concentration. The exchange activity showed saturation and was well described by Michaelis-Menten kinetics; the kinetic parameters derived are Vₘₐₓ of 19.6 ± 2.7 nmol × min⁻¹ per mg protein and Kₘ of 1.95 ± 0.45 μM (Fig. 5a). Clearly, the exchange activity in the gills is a powerful mechanism for Ca²⁺ transport, especially when cytosolic calcium concentrations exceed 1 μM (see discussion below; Fig. 6a).

The regulation of the Na⁺/Ca²⁺ exchanger activity in the gills has, until now, not received attention in our research. Studies on the effects of seawater adaptation of tilapia on the exchanger and its sodium dependence are in progress; effects of prolactin(s) and growth hormone on plasma membrane Na⁺/K⁺-ATPase (on which the exchanger depends) will be reevaluated in this respect.

What, then, is the role of the Na⁺/Ca²⁺ exchanger in the calcium homeostasis of the branchial cells? We have plotted the activities of the two plasma membrane calcium extrusion mechanisms as a function of cytosolic calcium levels (Fig. 6a). For the exchange activity, it was assumed that the Na⁺ gradient was not limiting its activity. At cytosolic Ca²⁺ concentrations up to 1 μM, the Ca²⁺-ATPase
There is limited data available on effects of calcitropic hormones on the intestine of fish. Takagi and coworkers ('85) successfully removed corpuscles of Stannius from trout and showed effect on intestinal calcium transport. Studies by Hirano ('89) have given evidence that STC in the eel controls the passive entry of calcium into the intestinal epithelium. In our lab we have shown that an extract of corpuscles of Stannius inhibits plasma membrane adenylate cyclase in tilapia enterocytes (Flik, '90), but this effect is likely to be attributed to the teleocalcin present in this preparation. However, recently we have been able to show a consistent stimulation by STC of IP3 production in tilapia enterocytes (P.M. Verbost, personal communication). Thus the enterocyte is a likely STC target and the presence of SMOCs in this cell should be further studied. For prolactin effects on the intestine of tilapia, indirect evidence was advanced (Flik et al., '86c): ovine prolactin reduces calcium loss via intestinal (and renal) routes.

One of the aims of our present research is to fill in the model of the endocrine regulation of Ca^{2+} transport in the enterocyte in analogy to the chloride cell of the gills. The intestinal epithelium has some important advantages over the branchial epithelium to make it an attractive model. The tilapia intestinal epithelium is easily stripped of its submucosa and was successfully used in an Ussing chamber setup (Flik et al., '90b; Van der Velden et al., '90). Furthermore, the epithelium is rather homogeneous as it contains no crypts. In biochemical studies, the enterocyte is easily fractionated to allow the isolation of brush border membranes (STC dependent SMOCs) and basolateral membranes (vitamin D and prolactin dependent extrusion mechanisms). We analyzed the calcium transport phenomena described here for the gills (earlier) in the intestine of the tilapia (Flik et al., '90b) and found some remarkable differences between these tissues. Cell mediated calcium transport in the intestine is dependent on the sodium status of the epithelium and it is Na^{+}/K^{+}-ATPase-dependent, as indicated by its sensitivity to ouabain. We subsequently analyzed the basolateral plasma membranes for Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchange activities. First we found an extremely low Ca^{2+}-ATPase mediated calcium transport in plasma membrane vesicles (K_{0.5} = 27 nM; V_{max} = 0.63 nmol x min^{-1} per mg protein). The low activity could not be attributed to a low quality of the membrane preparation but appears to indicate a low density of Ca^{2+}-ATPase therein. Surprisingly, a very high Na^{+}/K^{+}-ATPase could be demonstrated in these
Fig. 5. Na⁺/Ca²⁺ exchange in gills and intestine. Ca²⁺ dependence of Na⁺-driven Ca²⁺ uptake into vesicles of gill epithelium (a) and intestinal epithelium (b). Initial rates of Na⁺-driven Ca²⁺ uptake (Na⁺/K⁺ₐₒ) were corrected for "nonspecific" uptake (Na⁺ = Na⁺₀ = 150 mM). Ca²⁺₀ was varied between 10⁻⁶ and 2.5 × 10⁻⁵ M. Uptake rates have been plotted relative to the maximum velocities observed for comparison (Vₘₓ = 12.2 ± 3.7 and 20.5 ± 4.4 nmol x min⁻¹ per mg protein for gills and intestine, respectively). The Kₘ values are given in the figures. Mean values for six fish are given. Bars indicate SE.

membranes (specific activity around 150 μmol P_i × h⁻¹ per mg protein versus 30 μmol P_i × h⁻¹ per mg protein in a comparable plasma membrane preparation of gill cells). The high Na⁺/K⁺-ATPase activity also argues against a low quality preparation. However, a powerful Na⁺/Ca²⁺ exchange activity (K₀.₅ = 181 nM; Vₘₓ = 7.2 nmol x min⁻¹ per mg protein) was present in the plasma membranes of the enterocytes (Flik et al., '90b). The maximum activity of the exchanger exceeds that of the Ca²⁺-ATPase in the same membrane more than tenfold and that of any known Na⁺/Ca²⁺ exchange activity in (mammalian) non-excitable cells. An observation not understood at that time was the curvilinear calcium dependence of the exchanger (Flik et al., '90b), but very recently we have been able to solve this problem by improving on the procedures for calculation of free Ca²⁺ concentrations in physiological solutions (Schoenmakers et al., '92a,b). The improved calculation method
This contention is in good agreement with the Na⁺ dependence of calcium absorption in the intestine of this fish (Flik et al., '90b). In the model presented in Figure 1, the dominant Ca²⁺ transport pathway in the enterocyte is indicated as route 2.

Among the intriguing questions arising is how the exchanger is regulated. The Na⁺ dependence of a Ca²⁺ extrusion mechanism couples calcium homeostasis directly to sodium homeostasis, at least for this tissue. The endocrinological and physiological implications of this notion are of course manifold.

**MAGNESIUM TRANSPORT**

Magnesium is an essential element for all vertebrates, indispensable for proper functioning of all cells and involved in a variety of enzymic reactions. How magnesium is transported and how the transport is regulated is, essentially, an open question. We have studied Mg²⁺ transport phenomena in tilapia and carp. So far, special attention was paid to gills and intestine. The major impetus for the studies described below comes from the recent availability of reactor-produced Mg²⁺ radioisotopes, \(^{27}\)Mg²⁺ and \(^{28}\)Mg²⁺ (Van der Velden et al., '90; Kolar et al., '91).

**Gills**

The magnesium concentration in fresh water ranges mostly between 0.1 and 0.3 mM. For freshwater fish the ambient water thus represents an inexhaustible magnesium source (a similar reasoning holds true for calcium). Yet freshwater fish normally do not rely on absorption of Mg²⁺ from the water via their gills. When fed a low Mg diet, some fish (guppy, carp) may die even though Mg is present in the water (Shim and Ng, '88; Ogino and Chiou, '76); other species (rainbow trout, tilapia) survive such diets and even continue to grow (Knox et al., '83; Van der Velden et al., '91a). For the tilapia fed a low magnesium diet, we have calculated that the fish must absorb Mg²⁺ via its gills from the water to explain the growth observed (Van der Velden et al., '91b). However, we were unable to show any change in Mg²⁺ uptake from the water between control fish and low Mg fed tilapia (Van der Velden et al., '91a). For the carp, kept in water containing 0.2 mM Mg and fed a diet containing 31 μmol x g⁻¹ at maintenance level, the relative contribution of branchial magnesium uptake to the total magnesium uptake was calculated to be, at maximum, 16% (Van der Velden, '91). At least 84% is obtained from the food. For tilapia weighing 6.5 g, feeding on a control diet (25 μmol x g⁻¹), and kept in water containing 0.2 mM Mg, the con-
tribution of branchial \( Mg^{2+} \) uptake from the water was calculated to \( 13 \, \text{nmol} \times h^{-1} \), the contribution of drinking Mg containing water to \( 16 \, \text{nmol} \times h^{-1} \) maximally, and the contribution of Mg absorption from the food to \( 329 \, \text{nmol} \times h^{-1} \) maximally. For a fish feeding on a low Mg diet (1 \( \mu\text{mol} \times g^{-1} \)) these numbers are 12, 6, and 14 \( \text{nmol} \times h^{-1} \), respectively (Van der Velden et al., '91a; Van der Velden, '91). Clearly the tilapia does not increase extraintestinal \( Mg^{2+} \) uptake when fed a low Mg diet. In the latter situation, however, extraintestinal \( Mg^{2+} \) uptake stands for 50% of the total uptake!

For magnesium uptake in fish, the situation is opposite to that for calcium uptake: a not fully understood, small part of the uptake occurs normally via the gills, and the major route for \( Mg^{2+} \) uptake is via the intestine.

**Intestine**

There is ample evidence for absorption of \( Mg^{2+} \) by fish intestine. Many studies have given clear but indirect evidence by providing the fish with diets varying in Mg content and subsequently analyzing tissue elemental composition, growth rate, etc. A photonuclear reaction process has made available a preparation of \( ^{26}Mg^{2+} \) with very high specific activity and it allowed us to perform some studies on unidirectional \( Mg^{2+} \) fluxes in tilapia intestine (Van der Velden et al., '90). Net absorption of \( Mg^{2+} \) could be demonstrated and a sodium dependence established (Table 1). In control conditions with saline bathing the mucosal and the serosal sides of tilapia stripped epithelium, the net mucosa to serosa flux was \( 23 \, \text{nmol} \times h^{-1} \times cm^{-2} \). When sodium was replaced by the inert ion NMDG\(^{+} \) (sodium free condition), unidirectional fluxes were strongly reduced and the net flux was abolished. Ouabain, known to completely block \( Na^{+}/K^{+}-\text{ATPase} \) activity in this tissue (Flik et al., '90b), reduces the mucosa to serosa flux to the level found for the serosa to mucosa flux. Apparently this blocker inhibits the transcellular movement of \( Mg^{2+} \)

On the basis of analyses of the elemental composition of the epithelium and electrophysiological data (inside of the cell \(-60 \, \text{mV} \)) (Bakker and Groot, '88), we have concluded that intracellular \( Mg^{2+} \) (8 \( \text{mmol} \times 1^{-1} \)) is kept far below its equilibrium concentration (100 \( \text{mmol} \times 1^{-1} \); bath ringers Mg was 1 \( \text{mmol} \times 1^{-1} \)). It follows then that transport over the apical membrane may be passive and driven by an electrochemical potential difference of at least 25 mV. This value is probably too low, because the major part of the \( Mg^{2+} \) in the cell is in a bound form. Conversely, transcellular \( Mg^{2+} \) transport requires an energized extrusion step at the basolateral side of the cell to counteract the potential difference of at least 30 mV. Either ATP or the \( Na^{+} \) gradient over this membrane could provide the energy for such transport. In our Ussing chamber setup, the magnesium concentrations in both hemichambers were always identical. Also there is no electrical gradient across the epithelium in this setting. It follows then that the transport of \( Mg^{2+} \) must result from net water transport (solvent drag \( Mg^{2+} \) transport), from an active transport mechanism, or a contribution of both. The sodium dependence of the \( Mg^{2+} \) transport suggests, of course, the operation of an \( Na^{+}/Mg^{2+} \) exchanger. The latter hypothesis was tested in studies with tilapia enterocyte basolateral plasma membrane vesicles in which \( Mg^{2+} \) transport was determined with \( ^{27}Mg^{2+} \) as radiotracer (Kolar et al., '91). So far, we have been unable to determine \( Na^{+}/Mg^{2+} \) exchange in assays which were comparable in their setup to those for the determination of \( Na^{+}/Ca^{2+} \) exchange (Flik et al., '90b). However, a consistent finding so far is that ATP stimulates \( Mg^{2+} \) transport into these vesicles (Kolar et al., '91). A characterization of ATP-driven \( Mg^{2+} \) transport in plasma membrane vesicles has been carried out (Flik et al., submitted) and this finding will open a new field of research.

How magnesium transport in fish is regulated is still an enigma. STC appears not to be involved (Hirano, '89). External magnesium levels influence prolactin cell activity (Wendelaar Bonga et al., '85). Some further circumstantial evidence for a role of prolactin in magnesium homeostasis comes from diet experiments with tilapia: long-term low Mg diets result in chronically activated prolactin cells (Van der Velden et al., '91b). How prolactin affects magnesium transport is as yet unknown.

**TABLE 1. Magnesium fluxes (J) ± standard deviation across stripped intestinal epithelium of freshwater tilapia under different experimental conditions**

<table>
<thead>
<tr>
<th>Saline conditions</th>
<th>( J_{\text{max}} ) (( \text{nmol} \times h^{-1} \times cm^{-2} ))</th>
<th>( J_{\text{max}} ) (( \text{J}_{\text{max}} ))</th>
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<tbody>
<tr>
<td>Control</td>
<td>39 ± 19 (9)</td>
<td>16 ± 6 (8)</td>
</tr>
<tr>
<td>Sodium-free</td>
<td>6 ± 3 (7)</td>
<td>6 ± 2 (10)</td>
</tr>
<tr>
<td>+ Ouabain (1 mM)</td>
<td>16 ± 2 (3)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

1The number of observations are indicated between brackets. Data taken from Van der Velden et al. '90.

How magnesium transport is regulated is still an enigma. STC appears not to be involved (Hirano, '89). External magnesium levels influence prolactin cell activity (Wendelaar Bonga et al., '85). Some further circumstantial evidence for a role of prolactin in magnesium homeostasis comes from diet experiments with tilapia: long-term low Mg diets result in chronically activated prolactin cells (Van der Velden et al., '91b). How prolactin affects magnesium transport is as yet unknown.

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**LITERATURE CITED**


