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SHORT COMMUNICATION
MAGNESIUM TRANSPORT IN FISH INTESTINE

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Literature on the mechanisms of magnesium transport through fish intestine is scarce. Indirect evidence for a net magnesium transport *via* the intestinal tract of fish has been established by dietary experiments (e.g. with carp *Cyprinus carpio* L. and nile perch *Oreochromis niloticus* Peters), showing that dietary magnesium is essential for the growth of these fish (Ogino and Chiou, 1976; Dabrowska *et al.* 1989). Nakamura and Hirano (1986) suggested 'solvent drag' as a mechanism for net magnesium transport in eel *Anguilla japonica* intestine. For mammals, the reports on this topic are equivocal. In the small intestine of the rat, diffusion (Ross, 1962; Aldor and Moore, 1970; O'Donnell and Smith, 1973) as well as solvent drag (Behar, 1974) were reported to underlie magnesium absorption, although the possibility of an active transport component was kept open by these authors. For the intestine of guinea pig, active transport of magnesium was demonstrated (Ross and Care, 1962; Partridge *et al.* 1987). This communication deals with magnesium transport across stripped intestinal epithelium of freshwater tilapia and its possible mechanisms.

Male tilapia (*Oreochromis mossambicus* Peters), weighing around 150 g, were obtained from laboratory stock and kept in Amsterdam municipal tap water with a magnesium concentration of about 0.25 mmol l^{-1} and at a temperature of 28±2 °C. The photoperiod was automatically controlled (12 h light: 12 h dark) and the fish were fed once daily with tropical fish food (magnesium content 31 mmol g^{-1}; Tetramin). Fish were sacrificed by spinal transection just behind the gills. The intestine was cut free behind the stomach, transferred to saline and flushed with the same solution. Next, the intestine was cut lengthwise and the mucosa stripped of its underlying muscular layers as described by Albus *et al.* (1979). All experiments were performed at room temperature.

Key words: magnesium, freshwater teleost, *Oreochromis mossambicus*, ^{28}Mg, stripped epithelium, sodium, dependency.

The control saline contained (in mmol l^{-1}): NaCl (117.5), KCl (5.7), $NaHCO_3$ (25.0), NaH_2PO_4 (1.2), $CaCl_2$ (2.5), $MgSO_4$ (1.0) and mannitol (28.0); the saline was gassed with a mixture of 95% O_2 and 5% CO_2. In some cases ouabain (0.1 mmol l^{-1}) was added to the saline bathing the serosa. In some cases sodium was substituted by *N*-methyl-D-glucaminate ($NMDG^+$) adjusted to pH 7.4 with HCl (for NaCl), choline bicarbonate (for $NaHCO_3$) and KH_2PO_4 (for NaH_2PO_4). The extra addition of potassium in the form of KH_2PO_4 was compensated for by using 4.5 instead of 5.7 mmol l^{-1} KCl.

Segments of approximately 1 cm^2 of stripped intestinal epithelium from the proximal 15 cm of the intestine were fixed in a holder leaving an exposed tissue area of 0.2 cm^2. This holder formed the partition between two half-chambers, denoted as *a* and *b*. The set-up has been described in detail in Groot *et al.* (1979). The transport of Mg^{2+} was followed using $^{28}Mg^{2+}$ as radiotracer. The ^{28}Mg was produced by photonuclear reaction (Polak *et al.* 1989), yielding a specific activity of about 26 GBq mol^{-1}.

Prior to an experiment, both half-chambers were filled with 1.9 ml of magnesium-free saline. At time zero, 0.1 ml of a ^{28}Mg-containing $MgSO_4$ solution was added to half-chamber *a*, and 0.1 ml of $MgSO_4$ solution was added to half-chamber *b*. The resulting magnesium concentration in both half-chambers was 1 mmol l^{-1}, as confirmed by atomic absorption spectrometry (Perkin–Elmer 305). The contents of both half-chambers were constantly stirred and gassed. After 1 min, 0.5 h and 1 h, 0.5 ml saline samples were taken from half-chamber *b*. After each sampling, 0.5 ml of tracer-free 1 mmol l^{-1} magnesium-containing saline was added to the sampled half-chamber. At the end of the experiment (time 1.5 h) 0.5 ml saline samples were taken in triplicate from both half-chambers. The radioactivity of the samples was determined by liquid scintillation counting (Tricarb 2660, Packard Instruments).

The flux of magnesium, J_{ab} (in nmol h^{-1} cm^{-2}), to half-chamber *b* from half-chamber *a* was calculated using the equation:

$$J_{ab} = [R_b(t_2) - R_b(t_1)]Q_a/(t_2 - t_1)R_a(t_2)A\,,$$

where $R_b(t_2)$ and $R_b(t_1)$ are the radioactivities transported to half-chamber *b* (in cts s^{-1}) during times t_2 and t_1 (both in h), respectively; Q_a is the magnesium content of the 2 ml of saline in half-chamber *a* (in nmol); $R_a(t_2)$ is the radioactivity in half-chamber *a* at t_2 (in cts s^{-1}) and *A* is the exposed area of intestine epithelium (in cm^2). Since the flux might have been disturbed shortly after time zero because of adding the magnesium, we always based the calculation of the magnesium flux on the 1 h and 1.5 h data points. This assumption was justified as the tracer appearance rate proved constant with time over the total experimental period. The approximation given above for the calculation of the magnesium flux is only valid when the net flux is small and the total magnesium content of the 0.2 cm^2 epithelium is negligible compared with the content of magnesium in the saline in each half-chamber. This assumption was justified by our measurements (see below).

Three sets of experiments were carried out to determine the magnesium flux from mucosa to serosa (J_{ms}) and/or from serosa to mucosa (J_{sm}) in (i) control saline, (ii) sodium-free saline and (iii) saline with 0.1 mmol l^{-1} ouabain on the serosal side.

To determine the extracellular space and the elemental and water content of the stripped intestinal epithelium under the three experimental conditions, 1 cm^2 segments of stripped epithelium were pre-incubated for 15 min in 40 ml of control or sodium-free saline. Subsequently the segments were transferred for 1 h to 40 ml of fresh (experimental) saline containing 110 nmol l^{-1} hydroxy-[^{14}C]methyl inulin (Amersham) with a specific activity of 575 GBq mol^{-1}. All salines were continuously stirred by a flow of humidified gas (95 % CO_2+5 % O_2). Upon completion the segments were removed, blotted on moist filter paper (Whatman no. 1), weighed, dried to constant mass at 70 °C and weighed again. The dried tissue was extracted for at least 2 h in 0.1 mol l^{-1} HNO_3 and the extract divided into samples for the determination of [^{14}C]inulin by liquid scintillation counting and of the Mg, Na and K concentration by atomic absorption spectrometry.

The extracellular space, defined as the inulin space, was calculated as the ratio of the ^{14}C radioactivity per gram dry tissue and the ^{14}C radioactivity per ml medium. The intracellular space was calculated as the difference between tissue water content and extracellular space and was expressed in ml per gram dry mass of epithelium.

Data are presented as mean values±the standard deviation (S.D.). Only data points within a 95 % confidence interval (i.e. within two S.D. values) were taken for the calculation of the mean. Data were analyzed statistically using Student's *t*-test. Statistical significance was accepted at the 5 % level.

In the control situation, J_{ms} (39±19 nmol h^{-1} cm^{-2}; N=9) was significantly higher than J_{sm} (16±6 nmol h^{-1} cm^{-2}; N=8). A net magnesium influx across tilapia intestinal epithelium of about 23 nmol h^{-1} cm^{-2} was calculated. Replacement of Na^+ by $NMDG^+$ caused a drastic inhibition of J_{ms} (6±3 nmol h^{-1} cm^{-2}; N=7) as well as J_{sm} (6±2 nmol h^{-1} cm^{-2}; N=10). In the sodium-free saline there was no significant net magnesium flux. Addition of ouabain to the serosal saline decreased J_{ms} (16±2 nmol h^{-1} cm^{-2}; N=3) significantly.

Under control conditions, the epithelial water content was 4.2±0.6 ml g^{-1} dry mass stripped epithelium and the extracellular space was 3.4±0.7 ml g^{-1}; from these values we calculated a cellular water content of 0.8 ml g^{-1} dry mass epithelium (N=19). The epithelial Mg, Na and K concentrations (in μmol g^{-1} dry mass epithelium) were 27.9±4.1, 390±89 and 352±27, respectively. The calculated cellular concentrations for Mg, Na and K (in mmol l^{-1} cell water) were 7.9±1.6 (N=18), 87±14 (N=17) and 98±14 (N=16), respectively. Under sodium-free conditions, the cell water content decreased by 18 %; in the presence of ouabain it increased by 17 %. However, the Mg concentration of the total epithelium was not significantly affected. Under sodium-free conditions the cellular Mg concentration was 11.3±3.4 mmol l^{-1} (N=18), in the presence of ouabain it was 6.4±1.1 mmol l^{-1} (N=18). Under sodium-free conditions, the Na

concentration decreased by 69 % and the K concentration was not significantly affected. In the presence of ouabain the Na concentration increased by 91 % and the K concentration decreased by 67 %.

The dry mass of a 0.2 cm^2 tissue sample of stripped epithelium, as used in the flux experiments, was 0.46±0.14 mg (N=23). The mean total Mg content of 0.2 cm^2 stripped epithelium under control conditions was calculated to be 13±4 nmol.

In stripped intestinal epithelium of tilapia, net magnesium transport occurs from mucosa to serosa. Since the magnesium concentrations in both half-chambers are equal and there is no electrical gradient across the intestinal epithelium, the net magnesium transport will result from net water transport (solvent drag), from an active transport mechanism, or from both.

Studies on water fluxes across stripped intestine of freshwater teleosts have been performed for the Japanese eel (*Anguilla japonica*) and demonstrated a net flux of 5.5 $\mu l\, h^{-1}\, cm^{-2}$ in the middle section of the intestine (Ando and Kobayashi, 1978). The net water flux of non-stripped intestine was found to be considerably higher. In the same species of eel, Ando (1974) reported a net water flux of 16.5 $\mu l\, h^{-1}\, cm^{-2}$ in non-everted gut sacs *in vitro* (and thus in non-stripped epithelium). During an *in vitro* study with non-everted sacs of tilapia, Mainoya (1982) found a water flux across the anterior intestine of 0.44 $ml\, g^{-1}\, h^{-1}$. Assuming a wet mass of 10 $mg\, cm^{-2}$, we estimate a maximum net water flux for tilapia of 4.4 $\mu l\, h^{-1}\, cm^{-2}$ for a non-stripped epithelium. However, the value given by Mainoya (1982) was obtained in Ringer which contained glucose (this will increase the water transport *via* Na^+/glucose cotransport), and thus the value in glucose-free saline, as used in our study, was probably lower. Nevertheless, even if we proceed from this very high water transport rate in our set-up, a net magnesium flux of 4.4 $nmol\, h^{-1}\, cm^{-2}$ at most may be predicted *via* solvent drag. This value is considerably lower than the actual net flux of magnesium observed in our stripped epithelium. Therefore, we predict that solvent drag is not the major mechanism for net magnesium transport in the tilapia intestine.

Starting from the known cell potential of −60 mV in tilapia enterocytes (Bakker and Groot, 1988) and the saline Mg^{2+} concentration of 1 $mmol\, l^{-1}$ one may predict an equilibrium concentration in the cell of 100 $mmol\, l^{-1}$ Mg^{2+}. Our analysis of the tissues indicates a total concentration of about 8 $mmol\, l^{-1}$ magnesium. Thus, the magnesium uptake across the brush-border membrane could be passive and driven by an electrochemical potential difference of at least 25 mV. It is generally accepted that most of the intracellular magnesium will be bound. Thus, the intracellular Mg^{2+} activity in the enterocyte too will be much lower than the total cellular concentration, as determined, and therefore the inward driving force given above will be an underestimate. Conversely, transcellular transport requires energized extrusion at the basolateral membrane against an electrochemical potential difference of at least 30 mV.

The ratio of cellular K to Na found in this study is low. As noted earlier by Groot (1981), acclimation of fish to water of higher temperature results in a higher total

Na concentration in the enterocytes. However, it should be stressed that these numbers given refer to total Na and K concentrations and not to ion activities. The Na^+/K^+-ATPase of the tilapia enterocytes has a $K_{1/2}(Na^+)$ of 9 mmol l^{-1} (Flik *et al.* 1990) and this suggests strongly that the cellular $[Na^+]$ will be much lower (i.e. around 9 mmol l^{-1}) than the total sodium concentration (Pressley, 1988). As the cellular magnesium concentrations under the various experimental conditions differed only slightly, the measured fluxes may be compared. When Na^+ in the saline is replaced by the inert cation $NMDG^+$ (Palmer and Andersen, 1989) both unidirectional fluxes are reduced and the net magnesium flux is abolished. Addition of ouabain to the serosal saline, to block the Na^+/K^+-ATPase activity, reduced the flux from mucosa to serosa. Therefore, we postulate the involvement of a Na^+-dependent mechanism for active magnesium extrusion in the basolateral membrane of the enterocyte of freshwater tilapia. A possible mechanism is that of a Na^+/Mg^{2+} exchanger analogous to the Na^+/Ca^{2+} exchanger described for tilapia enterocytes (Flik *et al.* 1990). The presence of a Na^+/Mg^{2+} exchange mechanism has recently been demonstrated in chicken erythrocytes (Günther and Vormann, 1985) and squid giant axons (DiPolo and Beaugé, 1988). Lüdi and Schatzmann (1987), however, conclude that in human red blood cells magnesium extrusion is unlikely to utilize a Na^+/Mg^{2+} exchange mechanisms that depends on an inwardly directed Na^+ gradient. They suggest an ATP-energized extrusion mechanism that depends on external Na^+ for conformational translocation in the ionophoric part of the exchanger. Apparently, the mechanisms for magnesium transport differ among cell types and among species.

Further experiments using plasma membrane vesicles are presently underway to elucidate the underlying mechanism for net transepithelial magnesium transport in the intestine of tilapia.

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