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Intestinal zinc uptake in freshwater rainbow trout: evidence for apical pathways associated with potassium efflux and modified by calcium

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Abstract

Understanding the mechanisms of intestinal zinc uptake in fish is of considerable interest from both nutritional and toxicological perspectives. In this study, properties of zinc transport across the apical membrane of freshwater rainbow trout intestinal epithelia were examined using right-side-out brush border membrane vesicles (BBMV's). Extravesicular calcium was found to have complex actions on zinc uptake. At a low zinc concentration of 1 μM, calcium (0.1–2 mM) significantly stimulated zinc uptake. In contrast, calcium inhibited zinc uptake at higher zinc levels (100 μM). Lanthanum and cadmium in the external medium did not block zinc uptake, suggesting that interactions between zinc and calcium were not exerted at a calcium channel. Copper also failed to exercise any inhibitory action. Zinc association with the BBMV's was enhanced by an outward potassium gradient. This stimulatory effect was only present at a zinc concentration of 100 μM. The potassium channel blocker, tetraethylammonium chloride inhibited zinc uptake at this relatively high zinc concentration, suggesting the presence of a low affinity zinc uptake pathway linked to potassium efflux. The present study provides evidence that the mechanism of intestinal zinc uptake in rainbow trout is pharmacologically very different from that of the piscine gill and the mammalian intestine.

Keywords: Zinc; Fish; Dietary metal; Intestine; Calcium; Potassium channel

1. Introduction

The elucidation of zinc uptake pathways is critical to an understanding of the delicate balance that exists between maintaining an adequate zinc intake, and minimising the potential toxic effects of environmental zinc pollution. Zinc is an essential micronutrient [1], but is potentially toxic, particularly in the aquatic environment [2]. The uptake, and consequently the nutritional value and/or toxic effects of zinc, will be greatly influenced by the composition of the diet. In particular, the physicochemical similarity between zinc and other divalent cations, that are important as nutrients (e.g. calcium) and as toxicants (e.g. cadmium), predicts these entities may have a significant impact on zinc absorption [3]. Such competitive actions may be exerted at the site of uptake (e.g. the brush border membrane of the absorptive cells of the intestine [4]).

For fish, two major routes of absorption exist, the gut and the gills. Knowledge regarding the passage of zinc across the intestinal epithelium is limited in comparison to our understanding of branchial zinc uptake mechanisms. Zinc appears capable of entering the gills via an apical calcium channel located in the mitochondrial-rich, ion-transporting chloride cells (for review, see Ref. [5]). In contrast, the proteins responsible for achieving intestinal zinc uptake in fish have not been discerned at either a molecular or physiological level.

Information regarding competitive interactions between zinc and other cations in the alimentary canal of fish is sparse, but some evidence exists for an antagonistic interaction between copper and zinc [6,7]. This is consistent with investigations in the mammalian gastrointestinal system [8].

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Cadmium and zinc also interact in a competitive manner in mammalian intestine (e.g. Ref. [9]), while an inhibitory effect of luminal cadmium on subepithelial Zn(II) accumulation was described in perfused freshwater rainbow trout intestine [7]. A similar action was described in the winter flounder, (*Pseudopleuronectes americanus*), a marine teleost [6]. While both copper and cadmium have antagonistic effects on zinc uptake, the nature of their inhibition appears to be distinct, suggesting that these actions are exerted at separate loci [6,7].

A number of authors have reported decreased zinc absorption with increased calcium in the diet of fish (e.g. Refs. [10,11]). While the supplementation of dietary calcium as a phosphate salt, renders it difficult to distinguish between an effect due to calcium, or an effect mediated by a decrease in zinc bioavailability due to chelation by phosphate [10], evidence from in vivo perfusion studies of freshwater rainbow trout showed that equimolar calcium nitrate significantly retarded the passage of Zn(II) across the intestinal epithelium [7]. It is therefore possible that calcium and zinc share an uptake pathway in piscine intestine, similar to that of the gill, but in contrast to the gut of mammalian species where no antagonism between calcium and zinc is believed to exist [12].

There is evidence from the mammalian literature that other hydrominerals may also have direct influences over zinc transport. Investigations in a range of mammalian tissues have provided support for a zinc uptake mechanism linked to potassium efflux (e.g. Refs. [13,14]). Based on in vivo studies, physiological evidence was provided for a similar mechanism of zinc uptake in the rainbow trout intestine [7].

In the present study, brush border membrane vesicles (BBMV's) were used to examine intestinal zinc transport across the apical, brush border membrane of freshwater rainbow trout. This technique allows a mechanistic interpretation of zinc uptake across a well-defined step of the absorptive pathway, without the complication of mucus or higher integrative control from the animal itself [15]. Specifically, the modifying roles of calcium and potassium were investigated following in vivo reports that these cations influence zinc absorption [7], while the actions of other potential competing cations, copper and cadmium, were also studied.

2. Materials and methods

2.1. Animals

Adult rainbow trout (*Oncorhynchus mykiss*) were obtained from Houghton Springs fish farm in Dorset, UK. Fish were maintained in 300–400 l fibreglass tanks supplied with dechlorinated City of London tap water. Water temperature was maintained between 11 and 15 °C, varying with season. Fish were fed ad libitum three times a week on a diet containing 83 mg kg^{-1} Zn(II) (Mazuri Aquatic trout pellets).

2.2. Membrane collection and purification

Details of the methods used for collection and purification of the brush border membrane fraction have recently been described in detail elsewhere [16], and represent a modification of the protocol originally described by Pelletier et al. [17]. Briefly, membranes were isolated from scrapings obtained from the full length of the intestine removed from euthanised adult fish (~ 200–600 g). These scrapings were suspended in buffer 'A' (50 mM mannitol, 2 mM EGTA, 0.5 mM $MgSO_4$, 0.1 mM phenylmethanesulfonylflouride; pH 7.4), before homogenisation (20 strokes, Dounce homogeniser 'loose' pestle), and centrifugation (12,000 × *g*, 15 min; Beckman J2-21 refrigerated centrifuge, 4 °C) steps were performed. This yielded a white 'fluffy' layer that was resuspended in buffer 'B' (0.32 M sucrose, 10 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol (Tris; Sigma); pH 7.4), to which 10 mM solid $MgCl_2 \cdot 6H_2O$ was added to achieve membrane aggregation. A further cycle of homogenisation (25 strokes, Dounce homogeniser 'tight' pestle), and centrifugation (20,200 × *g*; 10 min; Beckman J2-21 refrigerated centrifuge, 4 °C; followed by 43,500 × *g*, 20 min; Beckman L8-80M Ultracentrifuge, 4 °C) were then performed, to yield the brush border membrane fraction. This pellet was resuspended in intravesicular assay buffer (see details in Experimental manipulations below) to a final protein content of 1 mg ml^{-1} by 30 passages through a 23-gauge needle [18], which also acted to vesiculate the membrane fraction. This protocol results in a right-side-out oriented, osmotically-sensitive brush border membrane vesicle fraction that is highly enriched and relatively free from contamination by other membrane fractions [16].

2.3. BBMV flux protocol

Zn(II) uptake by BBMV's was analysed by a rapid filtration method. Aliquots of BBMV's (35 μl; i.e. 35 μg of protein) were preincubated at 37 °C for 1 min. This incubation temperature was chosen to maximise transport rate, provide comparison with mammalian studies, and has been previously used successfully for BBMV experiments in rainbow trout [16]. Vesicles were then added to 125 μl of preincubated reaction (extravesicular) buffers. The composition of the reaction buffer varied according to the desired experimental condition (see Experimental manipulations below), but all contained ~ 62.5 kBq ml^{-1} ^{65}Zn(II) ($ZnCl_2$; Perkin Elmer), and were made up to the appropriate Zn(II) concentration by the addition of unlabelled $ZnSO_4 \cdot 7H_2O$.

For all treatments Zn(II) uptake was monitored as a function of Zn(II) concentration (1, 10, or 100 μM). It has been previously established that BBMV Zn(II) uptake is saturable with respect to time (see Fig. 3 in Ref. [16]), and therefore all data expressed herein are expressed as an initial rate (after 30 s). Incubation times up to 10 min were

examined for every condition tested, but followed the patterns observed after 30 s and are not reported here. Forty-five-microlitre aliquots of the reaction mixture were added in triplicate to 0.45 μM pore size methylcellulose membrane filters (ME25; Schleicher & Schüell) placed in a rapid filtration unit (Millipore). Filters were pre-soaked in ice-cold stop buffer (149 mM KCl, 1 mM NaCl, 0.5 mM EGTA, pH adjusted to 7.4 with Tris). Following the vacuum filtration of membrane vesicles, the filters were washed twice with 2 ml of ice-cold stop/wash buffer. Membrane filters were then counted for ^{65}Zn(II) activity (LKB Wallac, 1282 compugamma), with subtraction of non-specific filter ^{65}Zn(II) binding (residual ^{65}Zn activity in membrane-free controls).

Zn(II) uptake was calculated according to the following formula:

$$U = (\mathrm{cpm}/\mathrm{SA})/[p] \times t$$

where cpm is the background corrected counts per minute, SA is the specific activity (cpm nmol^{-1}), $[p]$ is the protein concentration in μg μl^{-1}, and t is time in minutes. This gives a Zn(II) uptake (U) expressed as nmol μg protein^{-1} min^{-1}.

In a previous investigation, the osmolality- and temperature-dependence of Zn(II) accumulation were determined in order to separate the binding versus transmembrane flux components of Zn(II) uptake in trout intestinal BBMV's [16]. Although the stop solution used herein contained EGTA, this is not considered to be entirely effective in displacing the bound Zn(II) component [16]. Consequently, the data reported here do not distinguish between Zn(II) that is actually transported across the apical membrane and that which may be bound by high affinity sites on the membrane surface. While this should be considered in the interpretation of the data presented, it should also be noted that both the binding and transmembrane components of uptake are likely to be of physiological importance.

2.4. Experimental manipulations

For all experiments the brush border membrane fraction was resuspended in a buffer consisting of: 149 mM KCl, 1 mM NaCl, 10 mM HEPES, pH 7.4. In control experiments the extravesicular buffer was identical, except for the addition of Zn(II) at 1, 10 or 100 μM. Geochemical speciation analysis (MINEQL+, Version 4.01; Environmental Research Software) showed that 77.5% of Zn(II) was present as Zn^{2+}, with little change in this proportion between experimental manipulations.

Zn(II) concentrations of 1, 10, and 100 μM were used to examine the effect of calcium (1, 10, 100, 1000, 2000 μM) on Zn(II) uptake. The influence of single concentrations of the competing cations lanthanum (10 μM; as $LaCl_3$), cadmium ($CdCl_2 \cdot 2\frac{1}{2}H_2O$), and copper ($CuCl_2 \cdot 2H_2O$) were also tested, at Zn(II) concentrations of 1, 10 and 100 μM. Consequently, for all divalent cations tested a range of divalent cation to Zn(II) ratios were examined.

A number of different manipulations were employed to investigate the importance of potassium efflux for Zn(II) uptake across BBMV's. In one set of experiments the standard extravesicular KCl assay medium described above was replaced with a NaCl-based assay buffer (149 mM NaCl, 1 mM KCl, 10 mM HEPES, pH 7.4), to provide assay conditions with a high intravesicular K^+ concentration, and a low extravesicular K^+ concentration, in contrast to the standard high K^+ on both sides of the vesicle. Accounting for dilution the final assay values for K^+ and Na^+ in the extravesicular buffer were 33 and 117 mM, respectively, compared to the corresponding intravesicular levels of 149 and 1 mM. Consequently, this provided an environment conducive for potassium efflux from the intravesicular space to the extravesicular medium, with limited change in net charge across the vesicle membrane. The opposite scenario was also tested wherein high intravesicular Na^+ (149 mM), low intravesicular K^+ (1 mM) were fluxed in the presence of high extravesicular K^+ (117 mM) and low extravesicular Na^+ (33 mM).

In a separate series of experiments, the inorganic potassium channel blockers magnesium and barium were added individually to the KCl-based extravesicular medium at a concentration of 10 μM. The influence of the organic potassium channel blockers tetraethylammonium chloride (TEAC; Sigma; 50 mM), and the membrane-permeant 4-aminopyridine (4-AP; Sigma; 10 mM) upon Zn(II) uptake was also examined. These entities were added to the intravesicular medium, and incubated on ice for at least 2 h before analysis of Zn(II) uptake was performed.

3. Results

At 1 μM Zn(II), calcium concentrations of 0.1 mM or greater (representing at least a 100-fold molar excess) significantly stimulated initial Zn(II) uptake rate (Fig. 1). For example, 2 mM calcium almost doubled Zn(II) uptake from 23.2 to 40.1 nmol mg protein^{-1} min^{-1}, a statistically significant increase ($P < 0.05$). However, as Zn(II) concentration increased to 100 μM, the stimulatory effect of calcium was replaced by inhibition of Zn(II) uptake. In the presence of 2 mM calcium, the Zn(II) uptake rate fell to 52 ± 8% of control (from 825.8 to 478 nmol μg protein^{-1} min^{-1}). While significant actions of calcium were again only observed at calcium levels of 0.1 mM or above, this represented an effective calcium concentration equimolar to the Zn(II) concentration.

The influence of lanthanum, copper, cadmium, barium, or magnesium on Zn(II) uptake was investigated by adding 10 μM of either of these metals to the perfusion medium also containing 1, 10, or 100 μM of Zn(II) (Figs. 2 and 3). Magnesium and barium both significantly inhibited BBMV Zn(II) uptake when in 10-fold excess to Zn(II) (Fig. 2). At

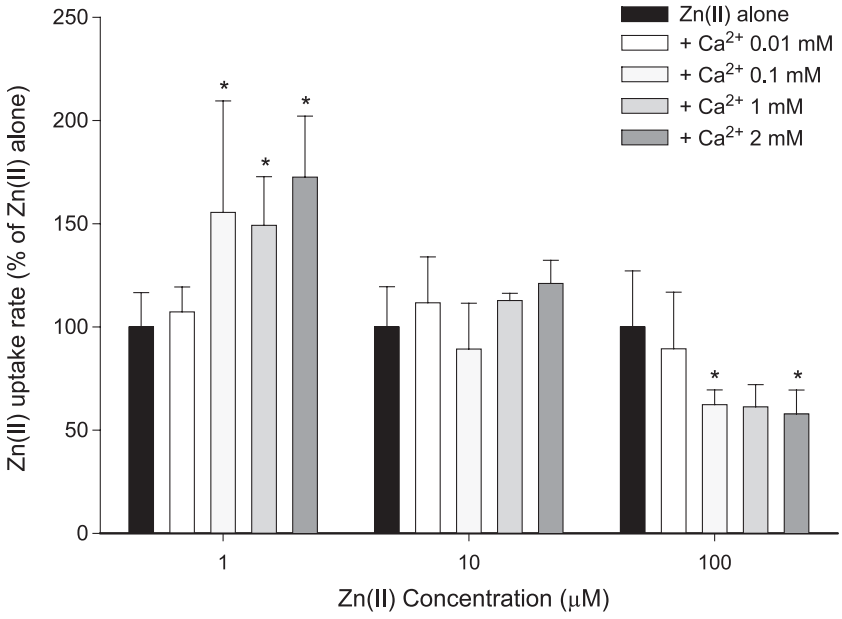

Fig. 1. Effect of extravesicular calcium on Zn(II) uptake rate into freshwater rainbow trout intestinal BBMV's, after a 30 s incubation at 37 °C. Rate is expressed as a percentage of control (Zn(II) alone) for presentation purposes only. Statistical significance ($P<0.05$, *) as determined by ANOVA and LSD analysis was performed on raw data. Plotted values represent the means ± S.E. of three to five calcium manipulations, and ten control (Zn(II) alone) experiments. Control (Zn(II) alone) values were 13.1, 103.0, and 825.8 nmol μg protein^{-1} min^{-1} at 1, 10 and 100 μM Zn(II), respectively.

higher Zn(II) concentrations, however, the inhibitory effect was diminished and not statistically significant. Neither cadmium or copper had any discernible effects upon Zn(II) uptake rate (Fig. 3). Addition of lanthanum enhanced Zn(II) uptake, but this was only statistically significant ($P<0.05$) when Zn(II) was present at 10 μM (Fig. 3). The stimulatory effect of lanthanum was not necessarily dependent on lanthanum being present in equimolar concentrations to Zn(II) because higher concentrations of lanthanum (1 mM) were tested and had an identical effect to that of the 10 μM lanthanum concentration (not shown).

Substitution of K^+ with Na^+ in the external assay medium resulted in a substantial and statistically significant increase

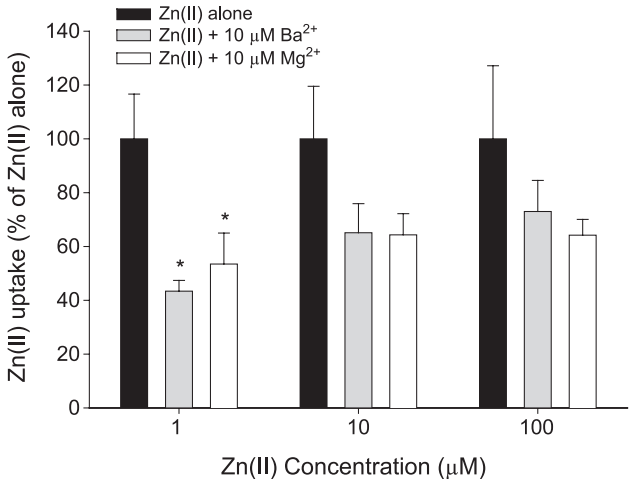

Fig. 2. Effects of extravesicular barium and magnesium (10 μM) on Zn(II) uptake into intestinal BBMV's after a 30 s incubation at 37 °C. Rate is expressed as percentage of control (Zn(II) alone), although statistical significance ($P<0.05$, *) was determined by ANOVA of raw data, followed by post hoc LSD analysis. Plotted values represent the means ± S.E. of four to five experiments for cation addition, and ten experiments for control data.

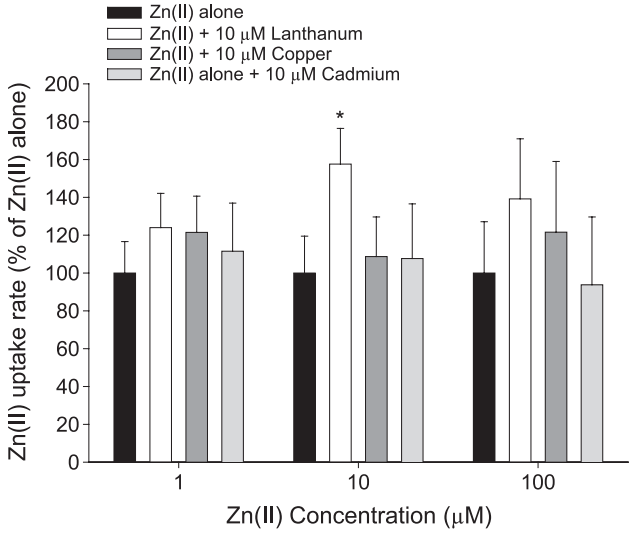

Fig. 3. Effects of extravesicular lanthanum, copper and cadmium (10 μM) on Zn(II) uptake rate into intestinal BBMV's after a 30 s incubation at 37 °C. Rate is expressed as percentage of control (Zn(II) alone), although statistical significance ($P<0.05$, *) was determined by ANOVA of raw data, followed by post hoc LSD analysis. Plotted values represent the means ± S.E. of four to five experiments for cation addition, and ten experiments for control (Zn(II) alone) data.

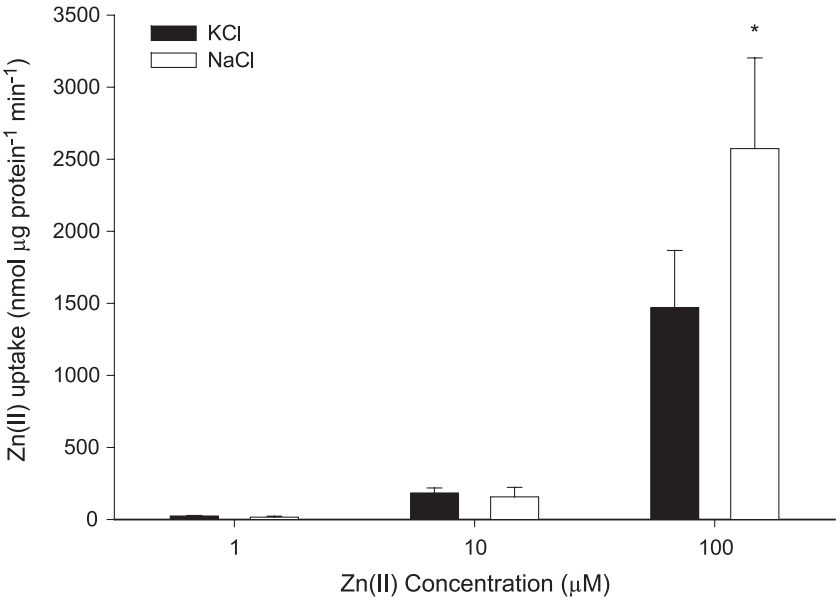

Fig. 4. Effect of extravesicular K^+ replacement by Na^+ on Zn(II) uptake rate (nmol μg protein^{-1} min^{-1}) into intestinal BBMV's after a 30 s incubation at 37 °C. Plotted values are the means ± S.E. of four (Na^+) or ten (K^+) experiments. Statistical analysis ($P < 0.05$, *) was determined by ANOVA, followed by post hoc LSD analysis.

in Zn(II) uptake at 100 μM Zn(II) (Fig. 4). At the lower Zn(II) concentrations of 1 and 10 μM, this stimulatory effect of extravesicular Na^+ was not observed. In experiments where K^+ and Na^+ gradients were reversed (i.e. intravesicular Na^+, extravesicular K^+), Zn(II) uptake was not different from the control situation (K^+ intravesicular and extravesicular; data not shown).

Because of the possible stimulatory effect of an outwardly directed K^+ gradient upon vesicular Zn(II) uptake, the effect of two K^+ channel blockers, TEAC and 4-AP, were investigated. At 100 μM Zn(II) TEAC, but not 4-AP, significantly decreased Zn(II) association with the brush border membrane (Fig. 5). The fall to 1036 nmol μg protein^{-1} min^{-1} from control values of 2021 nmol μg

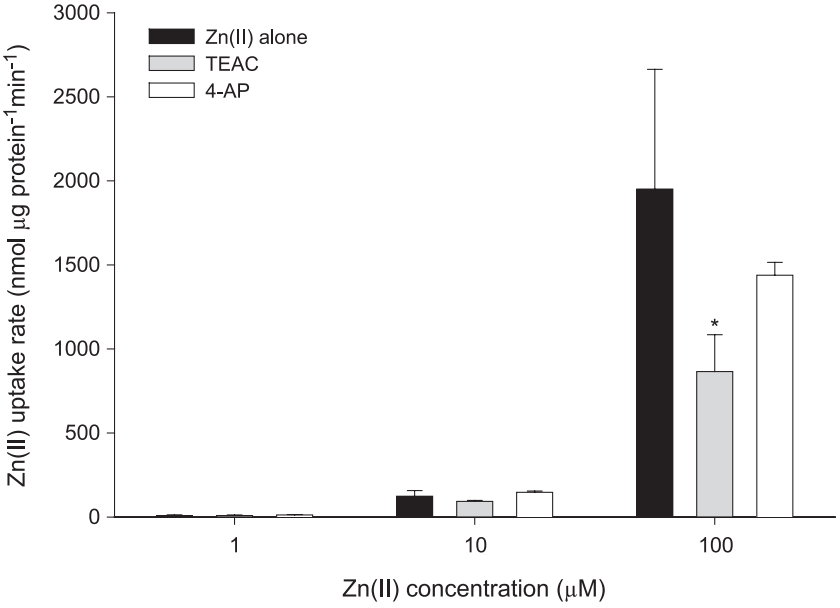

Fig. 5. Effects of the potassium channel blockers TEAC and 4-AP on Zn(II) uptake into intestinal BBMV's after a 30 s incubation at 37 °C, in the KCl-based extravesicular buffer. Plotted values are the means ± S.E. of four experiments. Statistical analysis ($P < 0.05$, *) was determined by ANOVA, followed by post hoc LSD analysis.

protein^{-1} min^{-1} represented a 49% decrease in vesicle Zn(II) accumulation ($P < 0.05$).

4. Discussion

4.1. Calcium-dependent Zn(II) uptake

The passage of Zn(II) across the brush border membrane of rainbow trout intestine appeared to be influenced in a complex, concentration-dependent manner by calcium. Calcium promoted Zn(II) accumulation at low Zn(II) concentrations (1 μM), but inhibited uptake at higher concentrations (100 μM). An inhibitory action of calcium on Zn(II) accumulation has been described in vivo for freshwater rainbow trout intestinally perfused with 50 μM Zn(II) [16]. The current analysis suggests that this effect was mediated at least in part, by an apical interaction between calcium and Zn(II). It should be noted that the maximum inhibition noted here was in the order of 40%, indicating the existence of both calcium-sensitive and calcium-independent mechanisms of Zn(II) uptake across the intestinal brush border of trout.

Zn(II) traverses the apical membrane of the piscine gill via a calcium channel (see Ref. [5]). The inhibitory effects of calcium in the current study, may have been mediated by a similar confluence of calcium and Zn(II) absorption. Two mechanisms for the passage of calcium across piscine intestine have been characterised. Biphasic calcium uptake across freshwater tilapia (*Oreochromis mossambicus*) intestine at concentrations less than 5 mM [18] was proposed to be an artefact of a low affinity calcium carrier operating considerably below J_{max} [19]. In a marine teleost, the Atlantic cod (*Gadus morhua*), calcium uptake was proposed to be mediated by a voltage-gated L-type calcium channel [20]. The inhibitory actions of barium and magnesium (Fig. 4) would support an effect on a calcium channel as both these ions are known to be weak antagonists of calcium channels [21,22]. However, lanthanum and cadmium, both of which strongly inhibit ion flow through calcium channels [22,23], and interfere with calcium and zinc uptake in rainbow trout gill (e.g. Refs. [24,25]), did not decrease BBMV Zn(II) uptake. The lack of any inhibitory influence of lanthanum or cadmium is not consistent with Zn(II) movement through a calcium channel in the rainbow trout intestine and thus implicates an interaction between calcium and Zn(II) at an alternative locus.

4.2. Cadmium and copper do not inhibit Zn(II) uptake

The fact that cadmium had no effect on apical Zn(II) uptake in the present study is not consistent with characterised zinc importers in other organisms. The family of Zrt-, Irt-like Proteins (ZIP) can mediate Zn(II) uptake in cells from organisms ranging from plants to man and characteristically exhibit inhibition of Zn(II) influx with cadmium and/or copper (see Ref. [26]). This would argue against involvement of ZIP in apical Zn(II) transfer in the rainbow trout intestine. It was recently proposed that ZTL1, a protein belonging to the cation diffusion facilitator (CDF) family, may mediate Zn(II) uptake at the human brush border membrane [27]. However, transporting properties of this protein are not fully described and it is not known whether or not it fits the pharmacological characteristics found here for Zn(II) uptake in trout BBMV's.

In vivo perfusion of Zn(II) in the presence of cadmium significantly altered the Zn(II) uptake profile in freshwater rainbow trout, but in accordance with the present study, there was no effect of cadmium on uptake of Zn(II) into the epithelium [7]. Instead, cadmium reduced the accumulation of Zn(II) in the muscle layers of the intestinal tissue, an effect that may not have been directly related to zinc transport across the epithelium. The current study is strongly indicative of a lack of interaction between the cadmium (Cd^{2+}) and zinc (Zn^{2+}) ions at the level of the brush border membrane. This is in contrast to cadmium-Zn(II) interactions in piscine gill, which are, at least in part, governed by apical competition effects [28]. In this regard branchial Zn(II) uptake in fish resembles that in the mammalian intestine. In mammalian gut, apical interactions between cadmium and Zn(II) have been well documented [8,9,29]. Therefore, Zn(II) uptake in piscine intestine exhibits notable differences in properties from that determined for the fish gill and mammalian intestine.

In the present study, copper, even when present in 10-fold excess, also had no significant effect on Zn(II) uptake across the apical intestinal membrane of freshwater rainbow trout. Conversely, at an identical Cu(II)/Zn(II) ratio, an inhibitory action of copper on Zn(II) uptake in a marine teleost intestine was observed [6]. Results from in vivo perfusion experiments in freshwater rainbow trout showed that copper reduced zinc accumulation in the post-intestinal compartments (i.e. whole body minus intestine [7]). Combined, these studies suggest that, in fish, Zn(II) uptake is inhibited by copper but the site of this inhibition is not the brush border membrane.

4.3. Stimulation of Zn(II) uptake at low concentrations by lanthanum and calcium: a displacement effect?

A surprising finding of the current study was the enhanced vesicular Zn(II) accumulation associated with lanthanum. A similar effect observed for Zn(II) uptake by rat glioma cells [30], was speculated to be caused by lanthanum inhibition of Zn(II) efflux [30–32], an explanation rendered unlikely due to a concomitant inhibition by lanthanum of Zn(II) influx [31,32]. The actions of lanthanum are instead likely be perpetrated by an interaction with the surface of the membrane [33]. The occupation of non-specific binding sites on the brush border membrane by lanthanum could exclude Zn(II)

from binding. This would effectively increase the concentration of Zn(II) in solution and enhance availability of Zn(II) for transport. It is probable that a similar competitive interaction also explains the stimulatory actions of calcium at the lowest (1 μM) Zn(II) concentration. This action was only observed when calcium was in 100-fold excess of Zn(II), a situation whereby calcium would be effective in raising local Zn(II) concentration, via this competitive displacement mechanism.

4.4. Zn(II) uptake linked to potassium efflux

Replacement of K^+ with Na^+ in the extravesicular medium stimulated uptake of Zn(II) at the highest Zn(II) concentration tested (100 μM). This condition created an inwardly directed Na^+ gradient as well as an outwardly directed gradient for K^+. Hence, it may be proposed that Zn(II) accumulation is linked to either of these gradients. Further experiments showed that the potassium channel blocker, TEAC, inhibited BBMV Zn(II) accumulation by 50%. A different potassium channel blocker, 4-AP, showed a lesser, statistically insignificant inhibition of Zn(II) uptake. This pattern (TEAC effective, 4-AP non-effective) is consistent with the pattern of inhibition observed with Ca^{2+}-activated potassium channels in excitable membranes [34]. Such a channel has been identified in the intestinal epithelium of the marine teleost fish, *Gillichthys mirabilis* [35]. Thus, a component of the brush border membrane uptake of Zn(II) might be linked to potassium efflux.

A potassium efflux-linked Zn(II) uptake mechanism is further supported by the observed inhibitory effects of barium and magnesium upon BBMV Zn(II) association. Barium has been shown to decrease serosal to mucosal potassium (rubidium) flux in flounder intestine, an effect attributed to an inhibitory action on a potassium channel [36]. Such an observation has also been made in the mammalian intestine [37]. Magnesium may also inhibit intestinal potassium conductance by a mechanism that involves channel blockade [38]. Zn(II) uptake through exchange with potassium has been indicated in a number of mammalian tissues (e.g. [13,14]). Such a mechanism of Zn(II) uptake across the freshwater rainbow trout brush border membrane is consistent with a previous finding that potassium in the intestinal lumen inhibits Zn(II) uptake in rainbow in vivo [7].

4.5. Biological significance

There is little literature documentation regarding Zn(II) levels in the intestine of wild-caught fish. Published values range from 18 μM in plaice (*Pleuronectes platessa*, [39]) to 300 μM in Atlantic salmon (*Salmo salar*, [40]). The Zn(II) requirement for rainbow trout in aquaculture has been estimated to 15–30 mg kg^{-1} dry pellet feed, which approximates 75 μM of hydrated food. Thus, the range of Zn(II) concentrations used in the present study (1–100 μM) were probably physiologically realistic and represented prandial luminal Zn(II) levels in diets ranging from Zn(II) deficient to sufficient. Likewise, the effects of calcium upon BBMV Zn(II) accumulation were observed at calcium concentrations (0.1–2 mM) likely to be physiologically relevant, with Shehadeh and Gordon [41] reporting an intestinal calcium concentration of 2.1 mM in freshwater rainbow trout. The results from the present study therefore suggest that calcium might enhance Zn(II) uptake from a Zn(II)-deficient diet, but would impair Zn(II) assimilation from diets that otherwise would be Zn(II)-adequate. These results might contribute to the low bioavailability of zinc from high-ash diets, which contain high levels of calcium [42].

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