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ORIGINAL ARTICLE

Effects of zinc ex vivo and intracellular zinc chelator in vivo on taurine uptake in goldfish retina

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Abstract Taurine and zinc exert neurotrophic effects. Zinc modulates Na^+/Cl^--dependent transporters. This study examined the effect of zinc ($ZnSO_4$) ex vivo and zinc chelator *N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine (TPEN) in vivo on [^{3}H]taurine transport in goldfish retina. The effect of TPEN in vivo on taurine and zinc levels was determined. Isolated cells were incubated in Ringer with zinc (0.1–100 μM). Taurine transport was done with taurine (0.001–1 mM) and 50 nM [^{3}H]taurine. Zinc (100 μM) noncompetitively inhibited taurine transport. TPEN was administered intraocularly and retinas extracted 3, 5 and 10 days later. Taurine was determined by HPLC (nmol/mg protein) and zinc by spectrophotometry ICP (mg/mg protein). Taurine and zinc levels decreased at 3 days and increased at 10 days after TPEN administration. At 10 days after intraocular TPEN, taurine transport affinity increased ($K_s = 0.018 \pm 0.006$ vs. 0.028 ± 0.008 mM). Apparently, zinc deficiency affects the taurine–zinc complex and taurine availability. The increased taurine uptake affinity by TPEN was possibly associated with a response to maximize retinal taurine content at low zinc concentration.

Keywords Retina · Taurine · Taurine transporter · Zinc

Abbreviations

DMSO	Dimethyl sulfoxide
i.o.	Intraocular injection
EC_{50}	Mean effective concentration
TPEN	*N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine

Introduction

Taurine (2-aminoethane sulfonic acid), a *β*-amino acid that contains a negatively charged sulfonic acid group, is present at high levels in the retina of many vertebrates (Militante and Lombardini 2002). This amino acid is known to be involved in the mediation of multiple functions, such as osmoregulation, modulation of calcium fluxes, neuromodulation, protection from oxidative stress, modification of protein phosphorylation, membrane stabilization, affectation of cell migration in the brain and the retina, regulation of axonal outgrowth, elevation in the number of regenerating retinal cells after nerve lesion and production of neural protection in certain neuropathies (van Gelder 1983; Lima et al. 1993; Law 1998; Lima and Cubillos 1998; Lima 1999; Nusetti et al. 2005). Thus, taurine possesses neuroprotective and neurotrophic properties in the central nervous system (CNS) during development and regeneration. For instance, during

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development of the retina, taurine favors maturation of the organized structure and preserves the visual function (Sturman et al. 1985). Some taurine functions are similar to those of zinc, an element with relevance to metabolic, genetic and neurotrophic processes (Vallee and Falcchuk 1993; Ahn et al. 1998).

Zinc is highly concentrated in the retina (Grahn et al. 2001; Ugarte and Osborne 2001). The physiological functions of zinc have been studied in the retina, where it is believed to interact with taurine, to modify photoreceptor plasma membranes, modulate synaptic transmission and serve as an antioxidant (Pasantes-Morales and Cruz 1984; Grahn et al. 2001). Zinc is essential for the normal development and function of the CNS and a deficiency of this element during early development can result in gross structural defects and pronounced behavioral abnormalities (Rogers and Hurley 1987; Oteiza et al. 1990; Gong and Amemiya 2001). Experimental evidence demonstrates that zinc can have both positive and negative effects on the cells, depending on the local concentration or state of the cell. An excess of free zinc can be harmful and trigger in vitro as well as ex vitro neuronal damage (Lees et al. 1990; Mazdai et al. 1991; Chai et al. 1999). This cation causes loss of the mitochondrial function, generation of reactive oxygen species (ROS), reduction in the production of energy and cell death (Weiss et al. 2000). The neuroprotective effect of low concentrations of zinc in the brain has been attributed to its antioxidant function and to the fact that zinc is an important modulator of excitatory synaptic transmission (Sandstead et al. 2000; Weiss et al. 2000; Ugarte and Osborne 2001). Zinc occurrence in cultures of goldfish retinal fragments at a concentration equal to or greater than 0.06 μM in the presence of taurine reduces the amino acid trophic effect, which could be related to the interaction between both in the membranes or to the influence of zinc on taurine transport. It has been demonstrated that micromolar concentrations of zinc modulate the activity of voltage-dependent calcium channels, receptors (Harrison and Gibbons 1994; Schetz et al. 1999; García-Colunga et al. 2005) and neurotransmitter transporters (Huszti et al. 2001; Scholze et al. 2002; García-Colunga et al. 2005). Taurine transporter, together with the dopamine, norepinefrine and serotonin transporters, modulated by zinc, form a part of a subfamily within the large family of the Na^+/Cl^--dependent transporters, all of which share the same topology of 12 transmembrane domains (Giros and Caron 1993). Previous results obtained by Lima et al. (2000) indicate that the trophic effect of taurine may be affected by the inhibition of its transport. Zinc, in concentrations equal to or greater than 0.06 mM, blocks the trophic function of taurine, which could be the result of transport inhibition. The evaluation of whether the presence or absence of zinc could affect taurine uptake was proposed as the objective of this work.

Materials and methods

Preparation of animals and cell suspension

Goldfish (*Carasius auratus*), measuring 3–4 cm, from a local commercial breeder (Fauna Roosevelt, Caracas, Venezuela), were kept in an aquarium in the laboratory under 12:12-h light cycle for 1–3 weeks before use. The fish were dark adapted for 30 min, which produces retraction of the retinal pigmented epithelium from the retina, and anesthetized with tricaine (0.05%) prior to enucleation of the eye. The eyes were rinsed in Lock's solution free of Ca^{2+} and Mg^{2+} and the retina was dissected, placed in the same solution with trypsin and incubated at 25°C for 30 min. The weight per retina was 30–35 mg and contained 1–2 mg of total proteins. Mechanical dissociation was performed gently with a Pasteur pipette. After centrifugation at room temperature with a swinging rotor in a Damon/IEC Division centrifuge at 600 *g* for 5 min, the pellet was washed and finally resuspended in Ringer solution (NaCl 125 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgSO_4{\cdot}7H_2O$ 1.3 mM, $Na_2HPO_4{\cdot}2H_2O$ 0.4 mM, NaH_2PO_4 0.1 mM, $NaHCO_3$ 25 mM, glucose 10 mM) (Guerra et al. 2000).

Kinetics of transport experiments

For determining experimental conditions, the uptake of 100 nM [^{3}H]taurine (891.7 GBq/mmol) into total retinal cells was measured in a preparation of approximately 250,000 cells per tube for different periods of time: 0.5, 2, 4 and 10 min to confirm the linearity of the uptake mechanism. The uptake of 100 nM [^{3}H]taurine was determined in dilutions of cell preparations of 100, 200, 300 and 500,000 cells per test tube. The cell preparation was preincubated at 25°C for 2 min in Ringer solution. Uptake was initiated by adding 100 μl of uptake buffer containing radiolabeled substrate. After incubation, the process was stopped by rapid filtration through fiberglass filters (Watman GF/B), followed by two washes with 5 ml of cold Ringer solution. The filters were placed in scintillation vials, dried and counted in 4 ml of toluene/omnifluor 0.4% in a Packard scintillation counter model Tricarb 1900 TR (efficiency 65–67%) (Guerra et al. 2000).

Saturation assays

The uptake of taurine was performed by isotopic dilution experiments with 50 nM [^{3}H]taurine and increasing concentrations of taurine from 0.25 to 300 μM, and in a preparation of about 250,000 cells per test tube to explore high- and low-affinity carriers. The transport was sodium dependent, and 80–90% inhibited by hypotaurine and beta-

alanine [50 mM, but not by gamma-aminobutyric acid (GABA)]. Finally, the saturation assays were measured by incubating the cell preparation (250,000 per tube) in the presence of various concentrations of [^{3}H]taurine from 0.01, 0.025 and 0.05 μM, followed by isotopic dilutions with concentrations of taurine from 0.05 to 1 μM and 50 nM [^{3}H]taurine. This concentration was chosen to target the high-affinity taurine uptake (Guerra et al. 2000). The preparation was preincubated at 25°C for 2 min in Ringer solution. Incubation was started by the addition of the substrate. After 5 min the process was stopped by filtration. The measurements were performed in triplicate.

Effect of zinc ex vivo on [^{3}H]taurine transport

The effect of zinc sulfate ($ZnSO_4$) was studied by preincubating the cell preparation with different concentrations of zinc, from 0.001 to 500 μM. The uptake was initiated by the addition of 50 nM [^{3}H]taurine plus 50 nM cold taurine. After 5 min of incubation at 25°C, the cells were washed and the process was stopped by filtration. Determinations were done in triplicates by liquid scintillation counting. This experiment was performed to determine the mean effective concentration (EC_{50}) of $ZnSO_4$. The mechanism by which $ZnSO_4$ affects taurine uptake was explored by high-affinity [^{3}H]taurine uptake in the presence and in the absence of different concentrations of $ZnSO_4$ (0.1, 1, 50, 100 μM).

Effect of *N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine in vivo on taurine transport

Goldfish received the intracellular zinc chelator, *N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine (TPEN), 2.5 nM in 0.31% dimethyl sulfoxide (DMSO) by intraocular (i.o.) injection with a Hamilton syringe (33 gauge needle); final concentration of TPEN and DMSO into the eye was 0.1 nM and 0.01%, respectively. The control group was treated only with DMSO, and the trauma effect of the needle was also tested. The retina was dissected, isolated and suspended in Ringer solution at 3, 5 and 10 days after the injection. Saturation assays were performed in isolated cells exploring the high-affinity [^{3}H]taurine uptake. Also, taurine and zinc levels in the retina were determined in all samples.

Amino acid analysis

Amino acids were determined by HPLC with fluorescent detection employing a modified method (Lima et al. 1998). The HPLC system consisted of a Waters 2690 Separation System and a Shimadzu RF-551 fluorescent detector. A Sulpeco LC-18 column 4.6 × 100 mm, 5 μm, was employed for amino acid separation. Each retina was homogenized in 300 μl of 0.4 M potassium borate buffer of pH 10.4 and 50 μl of 20% sulfosalycilic acid, and the aliquots were subjected to protein analysis (Lowry et al. 1951). Centrifugation of all tissue homogenates was carried out at 38,000 *g* for 20 min, at 4°C, and the supernatant was kept at 80°C for chromatographic analysis. Immediately before injection, 25 μl of the supernatants plus 175 μl of potassium borate buffer of pH 10.4 were derivatized by adding 200 μl of the following mixture: 25 mg o-phthaldehyde, 500 μl methanol, 25 ml *β*-mercaptoethanol (1 g/ml), and 4.5 ml 0.4 M potassium borate buffer of pH 10.4. Aliquots of the derivatized preparation were injected into the chromatographic system. Taurine was quantified by the method of the external standard and expressed in nmol/mg of protein using Millenium (Waters, MA, USA).

Determination of total zinc

Tissue samples were dried, suspended in 1 ml of concentrated HNO_3, heated for 6 h at 60°C and made up to 10 ml with deionized water. Inductively coupled plasma emission spectrometry (ICP-AES) was performed in a Perkin–Elmer Model Optima 3000 equipped with a U-5000AT ultrasonic nebulizer, a standard demountable-type quartz plasma torch and alumina injection (1.5 mm internal diameter). A ten-roller peristaltic pump was used to supply the gas with the sample solution (Lima et al. 2004). The atomic line for zinc was 213.856 nm.

Statistical analysis

Nonlinear fitting was done with the program PRISMA (GraphPad Prism 2). V_{max} and K_s of taurine transport were calculated either by Lineweaver–Burk plots or by curvilinear analysis. EC_{50} of $ZnSO_4$ was determined by curvilinear analysis. Each value was expressed as mean ± standard error of the mean (SEM). The probabilities of the differences among mean were derived from the analysis of variance (ANOVA), INSTAT 2 program (Tapia 1994). Significance was considered if $P < 0.05$.

Results

Cell preparation and incubation time

[^{3}H]Taurine uptake was linear during the 10 min of incubation at 25°C (Fig. 1). The incubation was fixed at 5 min for the rest of the experiments. The uptake was linear with respect to the number of cells between 50,000 and 400,000 per tube (Fig. 2). For all experiments, 250,000 cells were used.

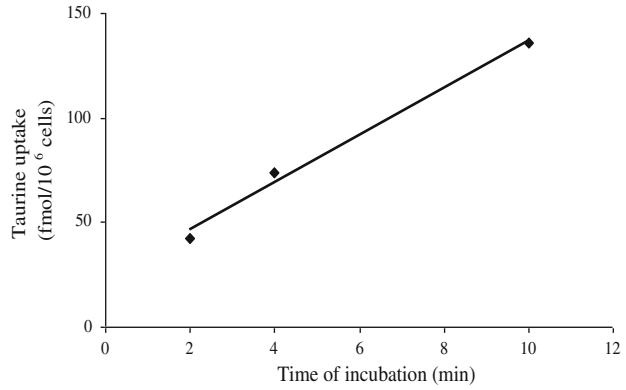

Fig. 1 Representative curve for the time-dependent [^{3}H]taurine (100 nM) into fresh isolated goldfish retinal cells

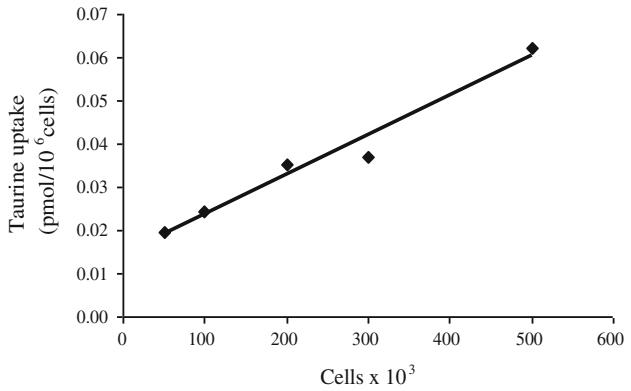

Fig. 2 Representative curve of [^{3}H]taurine transport (100 nM) into goldfish retinal cells in different cell preparations

Kinetic parameters of [^{3}H]taurine transport

The analysis of saturation experiments performed in the presence of 50 nM [^{3}H]taurine alone and with increasing concentration of non-labeled taurine from 0.25 to 300 μM resulted in a best fitting to a two-site model. The kinetic parameters of the high-affinity component were $K_{s1} = 0.020 \pm 0.003$ μM and $V_{max1} = 34.36 \pm 0.31$ fmol/l/10^6 cells; the low-affinity point component parameters were $K_{s2} = 5.29 \pm 0.21$ μM and $V_{max2} = 55.50 \pm 1.29$ fmol/l/10^6 cells. To work with the high-affinity component (Guerra et al. 2000), taurine uptake was measured in the 0.01–1 μM concentration range. The resulting saturation curve was hyperbolic, according to the Michaelis–Menten kinetic model (Fig. 3). Data obtained were transformed using the double reciprocal method, producing a linear graph with $R^2 = 0.94$. This was utilized to determine the kinetic parameters of the transporter, $K_s = 0.03 \pm 0.0056$ μM and $V_{max} = 38.72 \pm 2.29$ fmol/10^6 cells (Fig. 3).

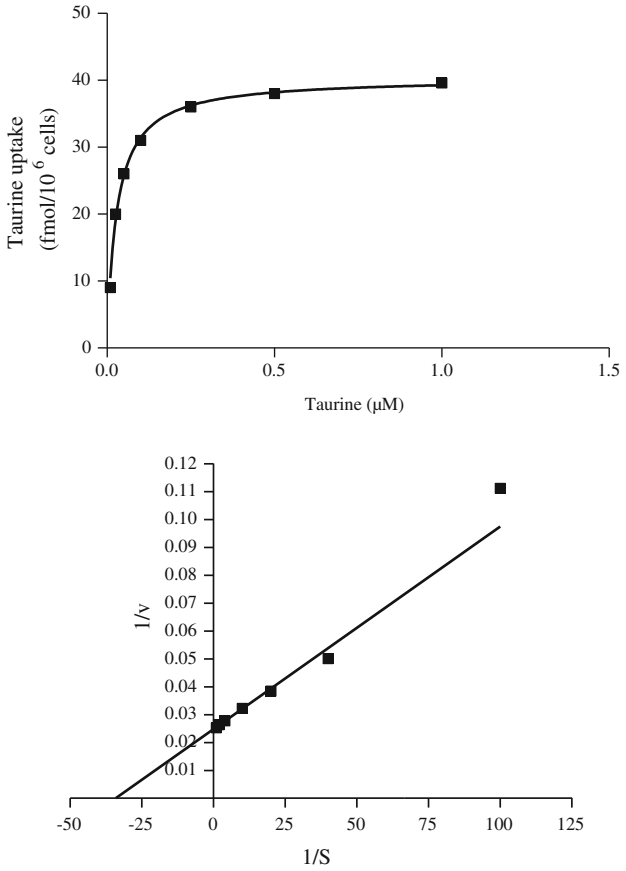

Fig. 3 Representative curve of the saturation of [^{3}H]taurine uptake into goldfish retinal cells determined by isotopic dilution. Data are best fitted to a rectangular hyperbola $R^2 = 0.96$. Lineweaver–Burk analysis of the same results, $R^2 = 0.98$

Effect of zinc ex vivo on [^{3}H]taurine transport

The simultaneous incubation of cell preparation with $ZnSO_4$ in concentrations ranging from 0.001 to 500 μM reduced the uptake with an $EC_{50} = 0.72 \pm 0.013$ μM (Fig. 4). The mechanism by which zinc affected high-affinity [^{3}H]taurine uptake was explored, at a zinc concentration of 100 μM. A 30% reduction in taurine transport capacity was observed, $V_{max} = 30.58 \pm 2.10$ fmol/10^6 cells, compared to the control group, $V_{max} = 39.72 \pm 2.02$ fmol/10^6 cells. At concentrations of 0.1, 1 and 50 μM of zinc, there were no significant modifications in either the V_{max} or K_s of taurine transport as compared to the control group (Table 1).

Effect of *N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine in vivo on taurine transport

No significant modifications were observed in the V_{max} and the K_s of high-affinity taurine transport 3 and 5 days after the i.o of TPEN compared to the control group (Table 2). Ten days after the i.o. of TPEN, a reduction of 40.82% in taurine transport affinity was observed, with a K_s of

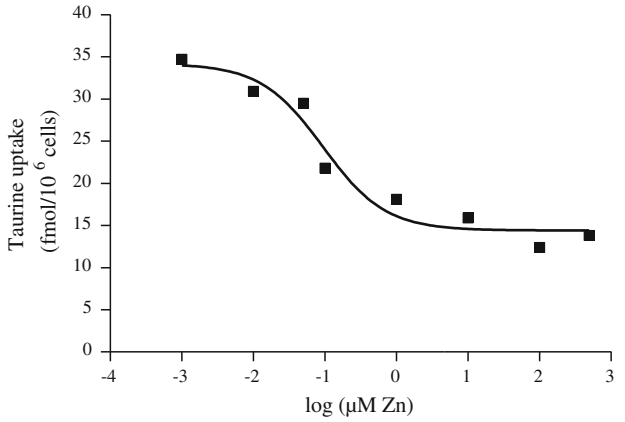

Fig. 4 Representative curve of the zinc inhibition of [^{3}H]taurine uptake in goldfish retinal cells

Table 1 Saturation experiments of high affinity of [^{3}H]taurine uptake in the presence of variable concentrations of zinc

Zinc concentrations	K_s (μM)	V_{max} (fmol/10^6 cells)
Control	0.030 ± 0.005	39.72 ± 2.00
0.1 μM	0.029 ± 0.002	40.88 ± 2.70
1 μM	0.027 ± 0.003	42.07 ± 5.10
50 μM	0.034 ± 0.002	35.35 ± 4.60
100 μM	0.027 ± 0.004	30.58 ± 2.00*

Each value is the mean ± SEM, $n = 3$

*$P < 0.05$ with respect to control

0.018 ± 0.006 μM compared to that of the control group, $K_s = 0.033 \pm 0.005$ μM (Table 2).

Effect of *N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine in vivo on taurine and total zinc levels in the goldfish retina

The concentration of total zinc after the i.o. of TPEN was significantly lower in 55% after 3 days and 62% after 5 days. At 10 days, there was a partial recovery, but the concentration was still 22% less than that of the control group (Fig. 5a). Taurine concentration was significantly lower at 3 days after the i.o. TPEN than that of control group and returned to values similar to those of the control 5 days after the injection (Fig. 5b). DSMO affected neither the zinc nor the taurine concentrations.

Discussion

Effect ex vivo of zinc on taurine transport in cells isolated from goldfish retinas

Taurine transport in cells isolated from goldfish retinas presented two components, one of high and another of low

Table 2 Saturation experiments of high-affinity [^{3}H]taurine uptake days after i.o. TPEN administration

i.o. TPEN	K_s (μM)	V_{max} (fmol/10^6 cells)
DMSO 3 days	0.028 ± 0.008	37.09 ± 4.50
TPEN 3 days	0.031 ± 0.007	38.72 ± 3.90
DMSO 5 days	0.030 ± 0.004	39.58 ± 6.40
TPEN 5 days	0.034 ± 0.006	40.51 ± 6.70
DMSO 10 days	0.033 ± 0.005	41.30 ± 4.60
TPEN 10 days	0.018 ± 0.006*	42.16 ± 2.10

Each value is the mean ± SEM, $n = 3$

*$P < 0.05$ with respect to DMSO 10 days

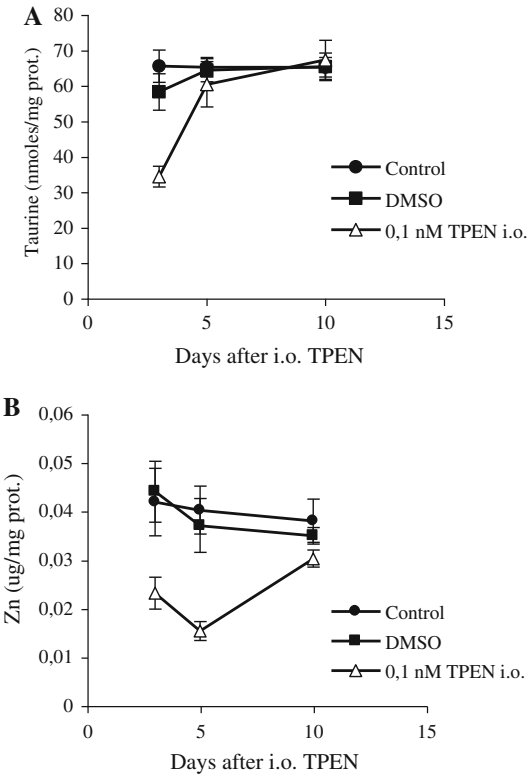

Fig. 5 **a** Taurine, and **b** total zinc concentration in the goldfish retina days after TPEN administration. Each value is the mean ± SEM, $n = 10$. *$P < 0.05$ with respect to control

affinity; these results agree with those obtained by Lima et al. (1991) and Guerra et al. (2000). The presence of two components of high and low taurine transport affinity has been demonstrated in cells isolated from rat retinas (Militante and Lombardini 1999), in pigment epithelial cells from both human and rat retinas (Salceda and Saldaña 1993; Grafe et al. 2004) and in human lymphocytes (Fazzino et al. 2006). In the present study, zinc caused a noncompetitive inhibition in high-affinity taurine transport, with $EC_{50} = 0.072$ μM. Taurine transport inhibition did not reach 50% in the concentrations used, possibly because

higher concentrations were needed to cause a greater inhibition. It may also be that greater inhibition was not observed because zinc affected only one taurine transporter, TAUT-1 or TAUT-2, and the unaffected transporter continued with the uptake. The mechanism by which zinc affects taurine transport has not been demonstrated. There may be a binding of zinc in the transporter that affects the union or translocation of the taurine, or possibly the formation of taurine–zinc complex, rather than the free zinc, which modulates the operation of the transporter. In fact, taurine transporter TAUT-1, taurine and zinc coexist in the ganglionic cells and in photoreceptors of goldfish and rat retinas (Nusetti et al. 2009). In other teleosts, such as zebra fish, zinc is also mainly localized in rod photoreceptors (Redenti et al. 2007).

Recent work has demonstrated that zinc differentially modulates Na^+/Cl^--dependent neurotransmitter transporters, such as those of monoamines (dopamine, norepinephrine and serotonin) (Scholze et al. 2002; García-Colunga et al. 2005) and other neurotransmitter transporters such as those of glutamate, histamine and GABA (Vanderberg et al. 1998; Huszti et al. 2001; Wu et al. 2006). Most of these studies coincide in that the effect of zinc, whether stimulation or inhibition, is direct on the transporter. Norregaard et al. (1998) demonstrated that zinc enhances the binding of cocaine analogs to the dopamine transporter, but that it is a noncompetitive inhibitor of dopamine transport in synaptosomal membranes (10 μM Zn inhibited the V_{max} by 30% and 1,000 μM Zn by 50%) and that the effect is because of the union of zinc in an area of the transporter that affects the translocation of the monoamine. Residues of the proteins that interact with zinc, His^{193}, His^{375} and Glu^{396}, which are close to one another (Loland et al. 1999; Norgaard-Nielsen et al. 2002), have been recognized in the tertiary structure of this transporter, and zinc facilitates the formation of oligomeric complexes in the transporter that affect the process of translocation of the neurotransmitter (Giros and Caron 1993).

It has also been reported that zinc inhibits the transport of glutamate in Müller cells and retinal cones of salamanders, with $IC_{50} = 0.84$ μM. The effect of the zinc was rapid and irreversible, which suggests a direct effect on the inhibition of transport (Spiridon et al. 1998). These authors conclude that 100 μM of zinc modifies the affinity of the transporter, as it seems that zinc does not compete for the binding sites of glutamate, sodium, potassium or for transporter protons, but instead binds to a point external to the membrane, which implies an allosteric modulation of the function of the transporter. Vanderberg et al. (1998) demonstrated that zinc is a noncompetitive inhibitor of the glutamate EAAT1 transporter expressed in oocytes of *Xenopus laevis*, with an $IC_{50} = 9.9$ μM and concluded on the basis of studies of site-directed mutagenesis that His^{146} and His^{156} residues of EAATI form part of the binding sites of zinc. These residues are found on the extracellular edge of the third transmembrane domain. Zinc bond alters the shape of this region, which reconstructs the contour of the pore, thus regulating the passage of the substrate and ions through the transporter.

Huszti et al. (2001) demonstrated that 100 μM of zinc sulfate ex vivo increases the uptake of the histamine neurotransmitter; the mechanism in this case is due to an increase of histamine binding sites (B_{max}) in membranes of astroglial and endothelial brain cells. It has also been demonstrated that zinc increases the serotonin uptake to a concentration of 1 μM in the corpus callosum cuts, although it remains to be seen whether or not it acts directly on the transporter (García-Colunga et al. 2005).

Zinc blocked the taurine transport in goldfish retinas with an $EC_{50} = 0.072$ μM. An ample range of effective concentrations of zinc has been reported regarding the effect of zinc on voltage-dependent calcium channels, with an $IC_{50} = 69$ μM, for *N*-methyl D-aspartate (NMDA), γ-aminobutyric acid (GABA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors, with EC_{50} of 0.1–100 μM, 0.6–320 μM and 300 μM, respectively, and for all dopamine and glutamate transporters with an $EC_{50} = 0.79$ and 0.84 μM, respectively (Harrison and Gibbons 1994; Kaneda et al. 2000, 2005). Takeda (2001) estimated that the concentration of extracellular zinc in the brain at rest is 10–20 nM; however, the concentration of extracellular zinc in the retina has not been reported, although if it is in this interval. Under these conditions, zinc does not affect taurine transport rate. As well as in other areas of the CNS, the labile zinc of the retina is liberated during neuronal activity, although it is possible that taurine transport could be inhibited when there is an excessive liberation of extracellular zinc, resulting in neurodegeneration. Yoo et al. (2004) demonstrated that a 15-min exposure to 300–500 μM of zinc resulted in significant neuronal death in cultured retinal cells.

The trophic effect of taurine was inhibited by micromolar concentrations of zinc in cultures of fragments of goldfish retinas. In addition, taurine did not counteract the inhibitory effect of 1 μM zinc (Nusetti et al. 2005), which may be due of inhibition of transport. This conclusion is consistent with the fact that the trophic effect of the taurine is dependent on the quantity of intracellular taurine (Lima and Cubillos 1998; González-Quevedo et al. 2003).

Zinc seems to have a neurotoxic effect through the activation of protein kinase C and inhibition of sodium potassium ATPase. The concentration used to obtain this effect was 300 μM during 15 min (Noh et al. 1999), a concentration three times higher and for a much longer

time than those obtained to inhibit taurine transport. Nevertheless, previous studies demonstrated that protein kinase C activators inhibit taurine uptake and affect the trophic function of the amino acid (Lima and Cubillos 1998).

The effect of zinc on glucose transport has also been evaluated. The incubation of adipose cells treated with 200 μM stimulates its uptake, although this stimulation is not from modulation of the transporter (12 transmembrane domains), but rather the result of the activation of insulin signaling channels, with an increase in the phosphorylation of insulin receptor in subunit β (Tang and Shay 2001) and of protein kinase B (AKT), and the inhibition of the enzyme kinase, 3β glycogen synthase (Ilouz et al. 2002).

Erikson and Aschner (2002) demonstrated that manganese, a metal which, like zinc, is highly regulated in the brain, inhibits glutamate transport and does not affect taurine transport, but does increase mRNA expression by 123% in cultures of rat astrocytes. It remains to be seen if zinc affects the expression of the taurine transporter in goldfish retinas.

Effect in vivo of *N*,*N*,*N*′,*N*′-tetrakis-(2-*pyridylmethyl*) ethylenediamine on taurine transport in cells isolated from goldfish retinas

Three days after the injection of TPEN i.o., a reduction in zinc and taurine levels in goldfish retinas was observed, which cannot be explained by the modification of the taurine uptake. It remains to be investigated whether or not the zinc deficiency, 3 days after administering the TPEN i.o., increases the taurine efflux. Wallwork and Sandstead (1983) found an increase in taurine excretion in the urine of rats with a zinc deficiency diet.

Ten days after TPEN i.o. injection, zinc levels in the retina were restored to approximately 80% and the taurine levels were similar to those of the control, showing an increase of taurine transport affinity. It would seem that low levels of zinc could affect the affinity of the taurine transporter, which could bind zinc to some site of the transporter modulating taurine affinity. This compensatory mechanism may increase the amino acid, with concomitant increases of zinc bioavailability in the retina. The study by Harraki et al. (1994) demonstrates that taurine produces a constant increase in zinc uptake in fibroblast cells and similar results were obtained in fish intestinal cells (Glover and Hogstrand 2002).

Ten days after injection of TPEN i.o., the taurine transport affinity increased in goldfish retinal cells and inhibited growth of nerve fibers in cultures of goldfish retinal fragments (Nusetti et al. 2006). Nevertheless, the latter effect was counteracted by taurine. It seems that the increase could be the reason for the reduction in the effect of zinc deficiency on the cultures of retinal fragments by taurine.

Taurine, taurine transporter and zinc have a neat distribution in the goldfish retina (Nusetti et al. 2009), although they do not co-localize exactly in this tissue, maybe indicating conjoint and also unique functions. For instance, rod retinal dysfunction in cats differs after taurine or zinc depletion (Jacobson et al. 1986). Specific zinc proteins have been located in cones and Müller cells of the human retina (Saghizadeh et al. 2009), and also related to ionotropic glutamate receptors in skate horizontal cells (Kreitzer et al. 2009), in which zinc, in addition to calcium and voltage, modulates hemi-gap junction channels in the rat (Sun et al. 2009).

Zinc levels are important to maintain an adequate concentration of taurine in goldfish retinas, possibly by modulating taurine transport. Zinc ex vivo and zinc deficiency in vivo have different effects on taurine transport; low zinc concentrations increase taurine uptake and high concentrations inhibit taurine transport. The mechanism, which exercises this effect, direct or indirect, over transport remains to be demonstrated.

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