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Modulation of taurine uptake in the goldfish retina and axonal transport to the tectum
Effect of crushing the optic nerve or axotomy

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Summary. Although there are a great number of studies concerning the uptake of taurine in several tissues, the regulation of taurine transport has not been studied in the retina after lesioning the optic nerve. In the present study, isolated retinal cells of the goldfish retina were used either immediatly after cell suspension or in culture. The high-affinity transport system of [^{3}H]taurine in these cells was sodium-, temperature- and energy-dependent, and was inhibited by hypotaurine and β-alanine, but not by γ-aminobutyric acid. There was a decrease in the maximal velocity (V_{max}) without modifications in the substrate affinity (K_m) after optic axotomy. These changes were mantained for up to 15 days after the lesion. The results might be the summation of mechanisms for providing extracellular taurine to be taken up by other retinal cells or eye structures, or regulation by the substrate taurine, which increases after lesioning the optic nerve. The *in vivo* accumulation of [^{3}H]taurine in the retina after intraocular injection of [^{3}H]taurine was affected by crushing the optic nerve or by axotomy. A progressive retinal decrease in taurine transport was observed after crushing the optic nerve, starting at 7 hours after surgery on the nerve. The uptake of [^{3}H]taurine by the tectum was compensated in the animals that were subjected to crushing of the optic nerve, since the concentration of [^{3}H]taurine was only different from the control value 24 hours after the lesion, indicating an efficient transport by the remaining axons. On the contrary, the low levels of [^{3}H]taurine in the tectum after axotomy might be an index of the non-axonal origin of taurine in the tectum. Axonal transport was illustrated by the differential presence of [^{3}H]taurine in the intact or crushed optic nerve. The uptake of [^{3}H]taurine into retinal cells in culture in the absence or in the presence of taurine might indicate the existence of an adaptive regulation of taurine transport in this tissue, however taurine transport probably differentially occurs in specific populations of retinal cells. The use of a purified preparation of cells might be useful for future studies on the modulation of taurine transport by taurine in the retina and its role during regeneration.

Keywords: Amino acids – Optic nerve – Retina – Taurine – Taurine axonal transport – Taurine uptake

Introduction

Taurine uptake systems have been characterized in several tissues and species (Huxtable, 1989; Lähdesmäki and Oja, 1973). It was first described that taurine uptake by synaptosomes from rat brain was sodium- and chloride-dependent and occured in high and low affinity manners (Chesney, 1985; Hruska et al., 1978; Kontro and Oja, 1978; Moyer et al., 1992). Taurine transport is mediated by the β-amino acid system, and is inhibited by hypotaurine (Adler, 1983; Holopainen et al., 1983), β-alanine (Larsson et al., 1986), guanidinoethyl sulfonate (GES) (Huxtable et al., 1979), and γ-aminobutyric acid (GABA) in astrocytes (Holopainen et al., 1988; Schousboe et al., 1976). However, in isolated synaptosomes GABA does not inhibit taurine uptake (Hruska et al., 1978), although it was proposed that in mouse brain, taurine, GABA and hypotaurine shared the same transport system (Kontro, 1981). The taurine uptake system is selective for β-amino acids, and energy and temperature dependency has to be determined in order to differentiate between binding and transport (Huxtable, 1989). The sodium- and chloride-dependent carrier, clearly demonstrated in the central nervous system (Hanretta and Lombardini, 1987; Kontro and Oja, 1978; Kontro, 1983), is also present in other tissues (Huxtable, 1989), including human placental brush-border membrane vesicles (Miyamoto et al., 1988).

Several investigators have reported a specific transport system for taurine (Heinämäki et al., 1986; Jayanthi et al., 1995; Kontro and Oja, 1978; Larsson et al., 1986; Pasantes-Morales et al., 1972; Petegnief et al., 1995; Starr and Voaden, 1972). However, there has been difficulty in the interpretation of results. Recently, molecular biology experiments have been very helpful in the understanding of structure, specificity and some aspects of regulation of taurine uptake. For instance, cloning of a high affinity taurine transporter from rat brain (Smith et al., 1992), mouse brain (Liu et al., 1992), and rat kidney (Uchida et al., 1992) has been performed and indicates that this carrier possesses homology to sodium- and chloride-dependent transporters, with twelve transmembrane domains. Moreover, the deduced protein sequence of mouse retinal transporter presents 93% homology with those of canine kidney, rat brain, mouse brain and human placenta (Vinnakota et al., 1997). In addition, the cDNA sequence of the taurine transporter is identical in human thyroid, placenta and retinal epithelium (Miyamoto et al., 1996).

Taurine can be taken up by neurons and glia (Assumpçao et al., 1979; Hösli and Hösli, 1980; Schousboe et al., 1976). The uptake of other amino acids, such as GABA (Kelly et al., 1974; Lascher, 1975) and several others (Sershen and Lajtha, 1976) have also been shown to occur in neurons and glia. In cell cultures, the glial transport of taurine increases with culture duration

(Borg et al., 1980). This effect also occurs in cortical neurons due to an increase in the maximal uptake rate (V_{max}) but without modifications in the apparent Michaelis constant (K_m) (Kuriyama et al., 1987). In developing mouse brain, the capacity and affinity of the high-affinity system decreases with age, but the low-affinity system does not (Oja and Kontro, 1984). Transport of taurine has been shown to decrease in neurons and increase in glia during maturation (Borg et al., 1980; Lähdesmäki and Oja, 1972). Reduction of the high-affinity component in different incubation conditions has been reported to be age-dependent in the mouse brain (Oja and Saraansari, 1996). Decrease of this taurine transporter also occurs in the cat brain during maturation (Saransaari and Oja, 1994).

Taurine plays a role in normal and degenerating neurochemical architecture of the rat retina (Fletcher and Kallonatis, 1996), as well as in the regeneration of the goldfish retina (Lima et al., 1988, 1998). In the rat with hereditary retinal degeneration, mainly affecting photoreceptors, the high-affinity component of taurine uptake is reduced (Schmidt and Berson, 1978). Moreover, the uptake of [^{3}H]taurine is higher in non proliferating areas of the retina, containing lower endogenous levels of taurine, than in proliferating ones (Lima et al., 1989).

The localization of taurine in the retina has been determined by autoradiography (Ehinger, 1973; Lake et al., 1978; Pourcho, 1977; Voaden et al., 1977), by antibodies (Lake and Verdone-Smith, 1989) or by conjugation with fluorescamine (Orr et al., 1976). These studies confirmed that taurine is more concentrated in the outer retina, mainly photoreceptors, but also located at the level of the inner layer in Müller cell processes. By *in situ* hybridization techniques, low levels of expression of this transporter have been demonstrated in the retina and pigment epithelium, and higher levels of expression have been demonstrated in the ciliary body of the mouse eye (Vinnakota et al., 1997). The bulk of injected radioactive taurine remains in the retina for more than 20 hours (Ehinger, 1973), indicating that the cellular transport of taurine is very efficient, concentrating the amino acid in the retina at a high level (Pasantes-Morales, 1986).

The trophic effect of taurine in the goldfish retina is reduced by inhibiting taurine uptake (Matus et al., 1997), also taurine concentration increases in the goldfish retina after lesioning the optic nerve (Lima et al., 1998). For these reasons, taurine uptake system in retinal cells is closely related to the stimulatory effect exerted by taurine on ganglion cell outgrowth. We previously demonstrated that taurine uptake occurs in retinal fragments of the goldfish by a saturable, sodium- and energy-dependent mechanism and with two kinetic components (Lima et al., 1991). In the present study, the high-affinity component was explored employing a narrow range of concentrations. The objectives were: 1) to characterize the high affinity taurine uptake system in isolated cells of goldfish retina, 2) to evaluate the possible modulation of this carrier by optic axotomy, 3) to study the capacity of the retina for taking up, storing, and transporting taurine during degeneration after crushing the optic nerve or axotomy, and 4) to explore the possibility of the regulation of taurine transport by taurine itself in primary cultures of retinal cells.

Materials and methods

Animals

Goldfish (*Carassius auratus*) measuring 4–5 cm from a local commercial breeder (Fauna Roosevelt, Caracas, Venezuela) and kept in an aquarium in the Laboratory under 12 : 12 h light cycle for a 1 to 3 weeks before were used for the study. Prior to surgery the goldfish were anesthetized with tricaine (0.05%). For all cell preparations the optic nerve of goldfish was sectioned (axotomy) or crushed with fine forceps and the animals were kept for several days before enucleation, dissection, and preparation of dissociated retinal cells.

Dissociated retinal cells and culture

The fish were dark-adapted for 30 min prior to enucleation of the eye. The eyes of goldfish were rinsed in Locke's solution free of Ca^{2+} and Mg^{2+} and the retina was dissected, placed in the same solution with 0.25% trypsin, and incubated at 25°C for 30 min. Mechanical dissociation was performed gently with a Pasteur pipette. The material was passed through a mesh. In most of the experiments, fresh retinal cells were utilized for measurement of [^{3}H]taurine uptake. In some experiments the cells were washed, resuspended (approximately 200,000 cells/ml) in nutrient Leibovitz medium (L-15, GIBCO, 2 ml per dish), plus 20 mM HEPES, 10% fetal calf serum, and 0.1 mg/ml of gentamicin (Sigma), and then incubated at 25°C for 24 hours to 8 days (Matus et al., 1997). Prior to plating on poly-L-lysine coated flasks, aliquots of each cell preparation were counted in a hemocytometer after staining with Trypan blue (viability, 90–96%).

Uptake experiments in isolated and in cultured cells

[^{3}H]Taurine (Amersham, 21 or 24.1 Ci/mmol, ≅200,000 dpm) was used in concentrations from 0.003 to 0.3 μM. Time-dependent and inhibition experiments utilizing β-alanine, hypotaurine, or GABA, 0.1 nM to 3 mM were performed in the presence of 40 nM taurine. The cell preparation (≅400,000 per ml) was preincubated at 25°C for 2 min in buffer solution. Incubation was started by the addition of the radioactive substrate. After 2 min the process was stopped by filtration through glass fiber filters (Whatman GF/B). The measurements were performed in duplicate with values within ±5% of the mean value. For cells in culture, 1, 3, 5 or 8 days, in the absence or in the presence of 4 mM taurine, the medium was removed and the flasks were washed with incubation solution. Refrigent cells were counted, [^{3}H]taurine was added to the attached cells, and incubation was performed for 2 min at room temperature. The process ended by removal of the incubation solution. The preparation was washed and 0.1 M NaOH, 1 ml, was added and maintained for 2 hours. An aliquot was then placed in a scintillation vial with Aquasol for counting radioactivity. The results are expressed in pmol or fmol of [^{3}H]taurine /10^5 cells.

Intraocular injection of [^{3}H]taurine

[^{3}H]Taurine, ≅70,000 dpm, was injected into the eye with a Hamilton syringe (33 gauge needle) in 2 μl of isotonic saline solution. Immediately after the injection, the optic nerve was exposed and either crushed with fine forceps or sectioned with scissors (axotomy). The retina, the optic tectum (Beltramo et al., 1994), and the optic nerve were dissected and isolated at 7 hours, 1, 3, 10 and 15 days after the lesion. Homogenization of the tissues was performed in distilled water with a Tissumizer (Tekmar Company, Cincinnati, OH). Aliquots of the homogenized tissue were counted in Aquasol in a Packard 1900TR scintillation counter (efficiency 60–65%). The results are expressed in dpm/mg of protein.

Statistical analysis

Analysis of variance and Student's *t*-test were performed with the program Primer of Biostatistics (Glantz, 1988). Statistical significance was considered if $P < 0.05$. Non-linear fitting was performed by Prisma 2.0. Kinetic constants, V_{max} and K_m, were calculated either by Lineweaver-Burk plots or by curvilinear analysis. Each value represents the mean ± S.E.M.

Results

Temperature, sodium and energy dependence of [^{3}H]taurine uptake

Incubation of retinal cells at 37°C increased the uptake of [^{3}H]taurine three times, while incubation on ice during 6 h significantly decreased [^{3}H]taurine transport compared to that at 25°C. The energy quotient, Q_{10}, was 2.18 ± 0.11. Substitution of lithium or *N*-methylglucosamine (NMG) for sodium ion decreased the uptake by 72% and 71%, respectively. The incubation of the cells in the presence of ouabain produced a reduction of 79% in taurine uptake (Fig. 1).

Kinetic analysis

[^{3}H]Taurine uptake in isolated cells from the goldfish retina was linear during the first 3 min of incubation at 25°C, and attained equilibrium by 5 min. The uptake was inhibited by β-alanine ($K_i = 0.10 \pm 0.04$) and hypotaurine (K_i =

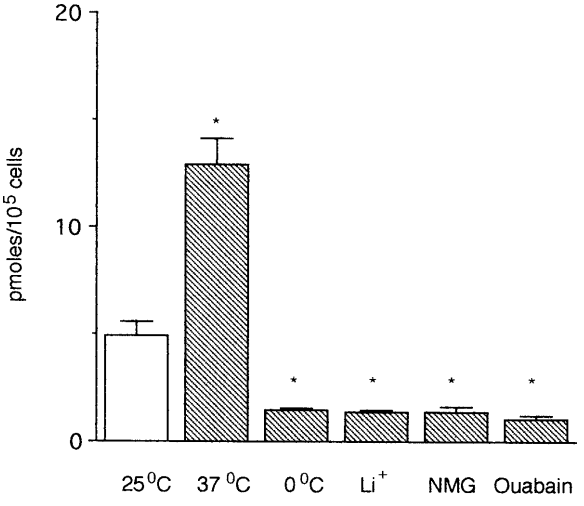

Fig. 1. [^{3}H]Taurine uptake into fresh isolated goldfish retinal cells under different conditions: incubated at 25 or 37°C during 2 min in the presence of sodium, incubated in an ice bath for 6 h, and incubated at 25°C in the absence of sodium and in the presence of same concentration of lithium, 240 mM *N*-methyl-glucosamine (NMG) or 10 μM ouabain. $F_{(5,18)} = 34.57$, $P < 0.001$. *$P < 0.05$ with respect to 25°C in the presence of sodium. N = 3–6

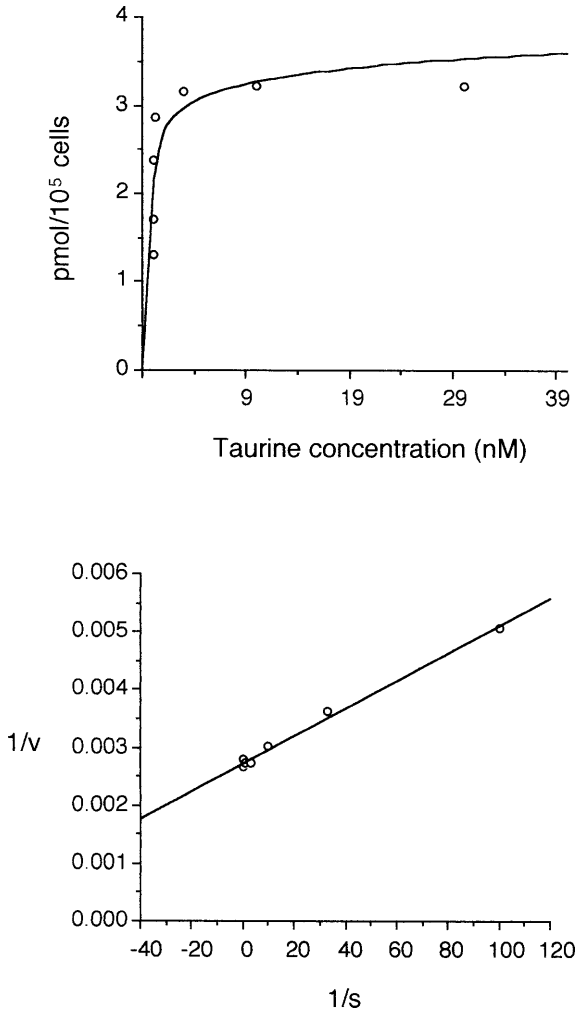

Fig. 2. Representative curve of the saturation of [^{3}H]taurine uptake into goldfish retinal cells 48 hours after lesioning of the optic nerve. Data are best fitted to a rectangular hyperbola $R^2 = 0.95$. Lineweaver-Burk analysis of the same results, $R^2 = 0.99$. Mean values are reported in Fig. 3

0.07 ± 0.02), but not by GABA. The high-affinity uptake was saturable and followed the shape of a rectangular hyperbola (Fig. 2). Data were transformed into Lineweaver-Burk plots to calculate the kinetic constants: $V_{max} = 5.23 \pm 0.28$ and $K_m = 7.71 \pm 0.76$ nM.

Effect of axotomy on [^{3}H]taurine uptake

Saturation experiments in control and post-axotomy retinas were performed. The V_{max} was lower at 1, 2, 5, 10 and 15 days post-lesion than in retinas from goldfish with an intact optic nerve (Fig. 3). There were no significant modifications in the affinity for the taurine substrate.

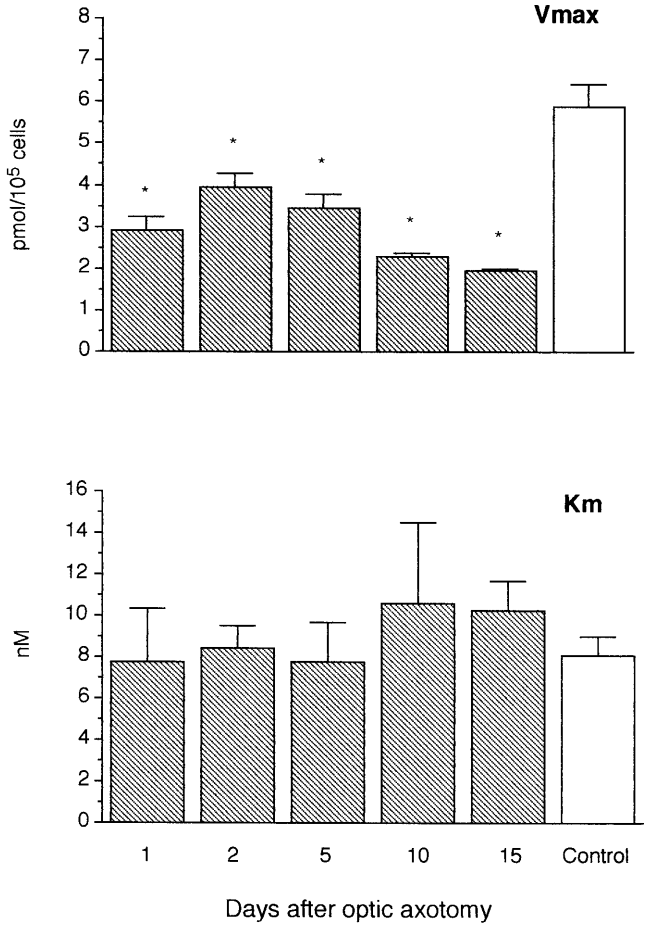

Fig. 3. [^{3}H]Taurine uptake into fresh isolated goldfish retinal cells from control animals in which the optic nerve was intact and several days after optic axotomy. V_{max}, $F_{(5,18)} = 12.17$, $P < 0.001$, *$P < 0.05$ with respect to control. K_m, $F_{(5,18)} = 0.39$, $P = 0.849$. $N = 6$

Modulation of [^{3}H]taurine uptake into retinal cells in culture by taurine

Retinal cells placed in culture for 2 days in the absence or in the presence of taurine transported [^{3}H]taurine with a significant lower V_{max} than cells cultured during 1 day. The uptake of [^{3}H]taurine measured at 5 and 8 days in culture in the absence or in the presence of taurine was not significantly different from that observed at 1 day. The K_m values, a measure of substrate affinity, was variable in the four groups, but there were no significant differences among them (Fig. 4).

In vivo time course of [^{3}H]taurine uptake in the eye

After injection of [^{3}H]taurine into the eye, radioactivity was determined in the retina, the optic tectum, and the optic nerve after optic nerve was crushed or after axotomy. Both treatments reduced the concentration of [^{3}H]taurine

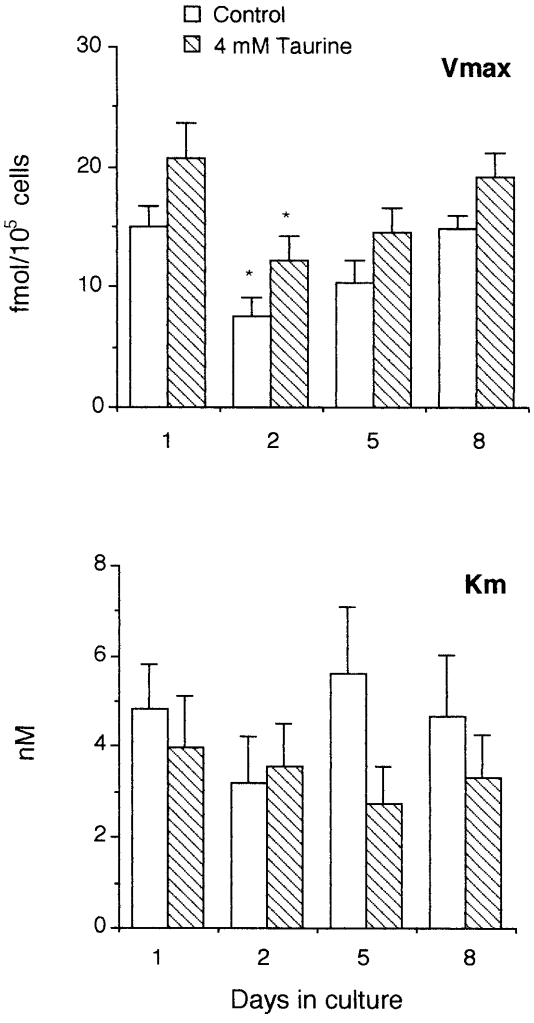

Fig. 4. Effect of the presence of 4 mM taurine in the culture medium of goldfish retinal cells from control animals in which the optic nerve was intact. The cultures were maintained for 1, 2, 5, and 8 days. In the absence of taurine: V_{max}, $F_{(3,20)} = 5.14$, $P < 0.01$, K_m, $F_{(3,20)} = 0.67$, $P = 0.578$. In the presence of taurine: V_{max}, $F_{(3,20)} = 2.52$, $P = 0.087$, K_m, $F_{(3,20)} = 0.28$, $P = 0.838$. *$P < 0.05$ with respect to corresponding 1 day. $N = 6$

during the first 3 days after the lesion as compared to controls, this decline in [^{3}H]taurine levels was greater after axotomy than after the optic nerve was crushed (Fig. 5). The half-life of [^{3}H]taurine in control retina was calculated approximatively, and were 7 days and 1–2 days in post-crushed optic nerve and post-axotomy, respectively. [^{3}H]Taurine concentration in the retina remained constant during the first 3 days in control retinas, but a significant decline in [^{3}H]taurine levels occured 1 days after injection in retinas in which the optic nerve was crushed. Following initial changes, the levels of [^{3}H]taurine remained constant up to 15 days after the lesion. The concentration of [^{3}H]taurine in retinas after the optic nerve was crushed was

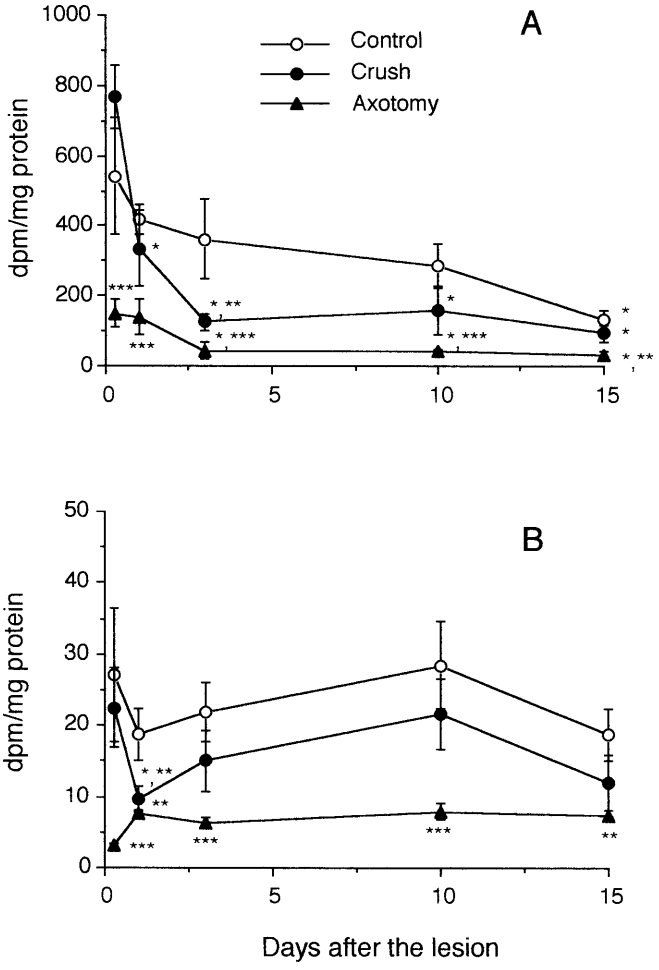

Fig. 5. Concentration of [^{3}H]taurine in the retina (A) and the optic tectum (B) of control goldfish and after the optic nerve was crushed or optic axotomy. [^{3}H]Taurine was administered as an intraocular injection. Retina: control, $F_{(4,23)} = 3.67$, $P < 0.05$; crush, $F_{(4,24)} = 10.28$, $P < 0.01$; axotomy, $F_{(4,30)} = 3.46$, $P < 0.05$. Optic tectum: control, $F_{(4,27)} = 1.61$, $P = 0.247$; crush, $F_{(4,10)} = 1.83$, $P = 0.200$; axotomy, $F_{(4,10)} = 2.13$, $P = 0.152$. *$P < 0.05$ with respect to 7 h after the injection in the same group, **$P < 0.05$ with respect to control at corresponding time after injection

significantly lower at 3 days after the injection of the tritiated amino acid as compared to the control retina. The accumulation of [^{3}H]taurine in the retina after axotomy was lower than that of control or after the optic nerve was crushed. The concentration of [^{3}H]taurine in the optic tectum was significantly different at 1 day after the optic nerve was crushed compared to the control. In the optic tectum of animals that had optic axotomy there was also a small accumulation of taurine, but significantly lower than in the control or post-crush retinas at 1, 3, and 10 days after the lesion. Intact or crushed optic nerves contained [^{3}H]taurine whose concentration decreased with time and was significantly higher in control than in lesioned nerves during the first days post-injection (Fig. 6).

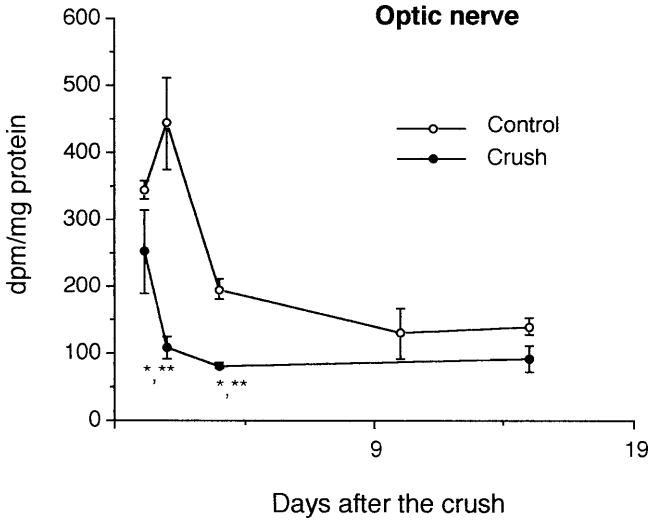

Fig. 6. Concentration of [^{3}H]taurine in the optic nerve of control goldfish and after the optic nerve was crushed. [^{3}H]Taurine was administered as an intraocular injection. Control, $F_{(4,10)} = 14.62$, $P = 0.001$; crush, $F_{(4,10)} = 5.61$, $P = 0.023$. *$P < 0.05$ with respect to 7 h after the injection in the same group, **$P < 0.05$ with respect to control at corresponding time after injection

Discussion

Transport of taurine into retina occurs through the pigment epithelium in the frog (Lake et al., 1977), babbon (Hussain and Voaden, 1985), and rat and cat (Schmidt, 1978), as well as in photoreceptors and Müller cells (Purcho, 1977), and in amacrine cells in mice and rats (Frederick et al., 1982). Taurine uptake in the retina is similar to that in the brain, although a wide range of values for kinetic parameters has been reported. Low- and high-affinity components are present and both are temperature-dependent (Huxtable, 1989; Pasantes-Morales, 1986). The main characteristics of the β-amino acid uptake system reported by several authors in different tissues and quoted throughout this work are also present in the preparation used for our experiments. It is of special interest that the remaining taurine uptake component observed in the absence of sodium, at low temperature, and by inhibiting Na^+-K^+-ATPase was about 20–30% of that at 25°C in the presence of sodium. The values of K_m for the high-affinity component are in the low μM range (Adler, 1983; Borg et al., 1976). In the preparation used for this study the concentrations of taurine employed were low and thus only the high-affinity uptake system was explored, which had a lower K_m. Other reports indicate that in human placenta the K_m is 6μM (Miyamoto et al., 1988), 4μM (Moyer et al., 1992), and 2.7μM (Ramamoorthy et al., 1993). In experiments for studying the regulation of taurine transport by protein kinase C (PKC) a concentration of 20 nM was used (Kulanthaivel et al., 1991).

Taurine is an essential amino acid during development, and a low plasma concentration of this amino acid in the fetus is caused by a reduced activity of

placental taurine transporters (Norberg et al., 1998). In addition, the concentration of taurine in fetal serum is around 500μM, which is 5 times greater than that of the mother (Malandro et al., 1996). Although the low-affinity component of taurine uptake in the retina could also be regulated, it is not surprising to find a high affinity regulated system, besides the elevated concentration of taurine in this tissue.

Most of the reports in the literature concerning the trophic effect of taurine indicate its role during development, and less attention has been given to the regeneration process. The concentration of taurine in the retina increases with age in the chick (Pasantes-Morales et al., 1973) and in the rat (Macaione et al., 1974). Taurine was found to be a component of the $<$1 kDa retinal fraction which stimulates rod development in rat retinal cultures (Altshuler et al., 1993). Moreover, these authors indicate that blockade of taurine uptake does not affect rod development, which might be an indication for the lack of a role for osmoregulation as a mechanism of action in this tissue. A few years ago it was demonstrated that pups nursed by mothers that received GES, an inhibitor of taurine transport, have low retinal levels of taurine and may be associated with visual dysfunction (Lake, 1983). It was also demonstrated that in taurine-deficient cats the transport of taurine increases in the retina (Schmidt et al., 1980), which suggests an adaptive regulation. Also newborn rats treated with GES showed severe disruption in photoreceptor structure (Pasantes-Morales et al., 1983). In addition, the capacity of intestinal cells in mice (Barada et al., 1997) and of retinal pigment epithelium (Salceda and Saldaña, 1993) to concentrate taurine decreases with age, which might be an indication of the need of taurine during early development. As it has been previously pointed out, decrease of taurine transport in neurons and increase of transport in glia were reported in cerebral hemispheres from newborn rats during maturation (Borg et al., 1980). However, in regeneration of the retina, as shown in the present study, the decrease in taurine uptake after lesioning the optic nerve is the result of transport into all types of retinal cells. We also report that the reduction of taurine uptake in the goldfish retina cannot be the result of cell death, since the data are expressed per number of cells with viability higher than 90%. However, the reduction in taurine uptake could be a mechanism for supplying taurine to retinal cells in order to act on the regenerating neurons or there could be a downregulation by the increase in taurine concentration (Lima et al., 1998).

Taurine transport has been shown to be regulated by neural inputs and muscular activity in rat skeletal muscle (Iwata et al., 1986), by illumination in the retina of the cat (Schmidt, 1978), by protein kinase C (PKC) in astrocytes (Tchomkeu-Nzouessa and Rebel, 1996a), in the glioma GL15 cell line (Tchomkeu-Nzouessa and Rebel, 1996b), by protein kinase A (PKA) and PKC in both *Xenopus* oocytes expressing the mouse retinal taurine transporter (Loo et al., 1996), and in Ehrlich ascitis tumor cells (Mollerup and Lambert, 1996), by adaptive regulation in two continuous renal epithelial cell lines (Jones et al., 1990), in *Xenopus* oocytes expressing poly (A) + RNA isolated from rat kidney cortex (Han et al., 1997), in the human placental

choriocarcinoma cells (Jayanthi et al., 1995), and in a human colon carcinoma cell line (Tiruppathi et al., 1992). There is special interest in the studies performed with Madin-Darby canine kidney (MDCK) cells, in which it was reported that when these kidney cells are switched to hypertonic medium the content of taurine doubles in accordance with the taurine concentration in the medium (Uchida et al., 1992). In addition, the abundance of mRNA encoding for the taurine transporter increases in these cells in hypertonic solution. This type of an adaptive response was also demonstrated in *Xenopus* oocytes expressing rat kidney mRNA after isolation of poly(A) + RNA from rats fed a low taurine diet, eliciting twice the uptake that is shown in oocytes injected with the RNA from rats receiving a normal taurine diet (Han and Chesney, 1994). Moreover, the adaptive response requires synthesis of RNA, since treatment with cycloheximide or actinomycin D prevents the adaptation of MDCK cells (Jones et al., 1994). Different types of regulation of taurine transport include hyper and hypoosmotic exposure of cultured rat liver macrophages, which respectively increases and decreases mRNA levels of the taurine transporter and the actual transport of taurine itself (Warskulat et al., 1997), and reduction of taurine transport in Caco-2 cells by dexamethasone (O'Flaberty et al., 1997).

The adaptive behaviour was previously reported in LLC-K1 cells of proximal tubular origin in which an increase in taurine transport was observed in taurine-starved cells (Jones et al., 1990). In primary tissue cultures the demonstration of adaptive regulation appears to be more difficult to demonstrate, especially if a mixed population of cells are used for the experiments. For instance, in goldfish retinal cells the ocurrence of adaptive regulation did not take place. However, even in the absence or in the presence of taurine there was a decrease in the uptake after 48 h in culture, probably due to the initial presence of taurine in the fetal calf serum. This result indicates a critical period of time for observing the regulation of taurine transport by the taurine substrate in this preparation.

It was previously demonstrated in rats that the migration of [^{35}S]taurine through the optic pathway does not correspond with extracellular or intracellular diffusion, rather it is transported axonally (Politis and Ingloglia, 1979), as was proposed for the goldfish visual system (Ingloglia et al., 1976). The fact that after the intraocular injection of [^{3}H]taurine into goldfish there was a decrease in radioactivity during the first 3 days in the retina in which the optic nerve was previously crushed as compared to the control retina. This result might be an indication of the death of certain type of cells. According to several reports, ganglion cells do not accumulate taurine (Pasantes-Morales, 1986), and after lesioning the optic nerve a gliosis might occur. It is unknown what type of cells store taurine for up to 4 days in the intact retina, but it appears that the tissue attempts to preserve the levels of the amino acid immediately after crushing the optic nerve. For instance, after optic axotomy in the goldfish, there is an increase of [^{3}H]leucine incorporation in ganglion cells, which has been related to an increase in protein synthesis (Murray and Grafstein, 1969). The lesion produced in the present experiments was mild, since crushing the optic nerve did not significantly reduce the transport to the

optic tectum. This might be an indication of an sufficient transport by the lesioned nerve through the remaining fibers or an increase through the fibers for maintaining specific functions in the optic tectum which are mediated by taurine. There are great differences in the outgrowth from goldfish retinal explants after crushing the optic nerve or after axotomy, the latter is a more potent stimulus for axonal sprouting (Cubillos and Lima, 1997). The contribution of extraxonal delivery of [^{3}H]taurine to the tectum is small, as is shown by the low accumulation of taurine in the tectum of goldfish which are sujected to optic axotomy. As a result of the higher number of retinal cell loss by axotomy there was a reduction in the accumulation of taurine in the retina, which was observed to be lower even a few hours after sectioning the optic nerve. Despite the demonstration of axonal transport from the retina to the optic tectum, ganglion cells have not been shown to accumulate taurine, but some mechanisms allow taurine to migrate to the tectum. The presence of taurine in control and crushed optic nerves is a demonstration of the neural delivery of taurine to the tectum, although the concentration of taurine was higher in intact nerves than in crushed nerves during the first days after the injection of [^{3}H]taurine. However, the concentration of taurine in the tectum was not significantly different between the two groups.

In summary, isolated cells from the goldfish retina possess a sodium-temperature- and energy-dependent high-affinity taurine uptake system inhibited by hypotaurine and β-alanine. Lesioning the optic nerve decreased V_{max} of taurine uptake without affecting K_m. This might be due to the increase in taurine levels previously reported to occur after crushing the optic nerve (Lima et al., 1989, 1998). In addition, the accumulation of taurine in the retina is lower after crushing the optic nerve, and considerably lower after axotomy, probably due to the differential reduction of cells. However, adaptive regulation might occur in the goldfish retina. To answer this question, more research must be done focusing on distinct cell populations.

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