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Functional Expression and Subcellular Localization of the Taurine Transporter TauT in Murine Neural Precursors

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Key Words
Neurospheres · Neural stem cells · Amino acids · Cell growth

Abstract
Taurine addition to cultured embryonic neural precursor cells (NPC) significantly increased cell proliferation [Hernández-Benítez et al., 2010]. The medium used for NPC growing and proliferation is a fetal serum-free medium, and therefore, NPC become taurine depleted. Addition of taurine to the cultured medium fully replenished the cell taurine pool, suggesting the functional expression of a taurine transporter (TauT) in these cells. In the present study, TauT in NPC was functionally characterized and its protein expression and the subcellular distribution of immunoreactivity were determined. ^{3}H-taurine uptake in NPC could be separated into a non-saturable component and a Na^+/Cl^--dependent, saturable component. The saturable component showed an apparent 2:1:1 Na^+/Cl^-/taurine stoichiometry, a V_{max} of 0.39 ± 0.04 nmol/mg protein/min, and a K_m of 21.7 ± 2.6 μM. TauT in NPC was strongly inhibited by hypotaurine and β-alanine (92 and 79%, respectively) and reduced (71%) by γ-aminobutyric acid. TauT protein is expressed in NPC as a single band of about 70 kDa. Essentially all (98.8%) of the neurosphere-forming cells were positive to TauT immunoreactivity. Immunolocalization visualized by confocal microscopy localized TauT predominantly at the cell membrane. TauT was also found at the cytosol and only occasionally at the nuclear membrane. This study represents the first characterization of TauT in NPC.

Introduction

Taurine is a sulfur β-amino acid present in most animal cells (in mM concentrations). It is not a protein constituent and is present largely free in the cytosol. The highest taurine levels are found in excitable tissues: heart and skeletal muscle, secretory glands, the brain and retina, where taurine levels of up to 60–80 mM have been reported [Huxtable, 1992; Wójcik et al., 2010]. Taurine is not involved in metabolic reactions in the cell, besides the synthesis of taurocholic acid in the liver, but it has a prominent role as an osmolyte in numerous cell types [Pasantes-Morales et al., 1998]. This function essentially involves its translocation through the membrane in the direction required to correct deviations in cell volume. Besides these physiological roles, taurine, when present

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in large amounts, has a variety of effects on different cell types. Modulation of Ca^{2+} homeostasis, antioxidant effects and cytoprotection are all well-documented taurine actions [Schuller-Levis and Park, 2003; Xu et al., 2008]. Taurine concentration in a cell type results from its ability to synthesize, its capacity to accumulate from the extracellular milieu and its turnover rate. Taurine synthesis is restricted to some cells and tissues, and the biosynthetic capacity varies between species. Taurine is formed from cysteine via a pathway requiring cysteine dioxygenase and cysteine sulfinate decarboxylase. The latter is a rate-limiting enzyme, and its presence and activity often reflect the ability of a given tissue to synthesize taurine. In the liver and kidney, taurine is actively synthesized while its synthesis is limited in the brain and absent in the heart and skeletal muscle [Huxtable, 1992; Stipanuk, 2004; Tappaz, 2004]. Taurine transport is of key importance to maintain the high levels typical of excitable tissues. Taurine is transported inside cells by a well-characterized and ubiquitous taurine transporter (TauT). TauT is able to concentrate taurine inside the cells at concentrations several orders of magnitude higher than the extracellular concentrations. In most cells of different types, TauT is a Na^+/Cl^- and energy-dependent carrier, transporting taurine with high affinity and low capacity and showing a narrow specificity for β-amino acids [Tappaz, 2004]. Knockout of the TauT gene in a mouse model shows a 98% decrease in taurine levels in skeletal and heart muscle, 80–90% in the brain and retina and 70% in the liver, stressing the key importance of taurine transport in maintaining physiological taurine tissue levels. The $TauT^{-/-}$ phenotype is characterized by severe and progressive retinal degeneration, reduced brain size and shrunken kidneys [Heller-Stilb et al., 2002; Warskulat et al., 2007].

In the adult mammalian brain, taurine is found at levels ranging from 1 to 13 mM, the highest found in the mouse brain [Huxtable, 1992]. Taurine levels are markedly high in the immature brain as compared with the adult brain [Rassin et al., 1981; Sturman, 1993; Miller et al., 2000]. This decline is a consistent feature observed among species, regardless of their differences in taurine concentration. A high taurine cell content may thus be a requirement for developing brain cells. Interestingly, the olfactory bulb, which maintains a constant neurogenesis in the adult, also maintains a high taurine concentration [Miller et al., 2000; Miranda-Contreras et al., 2000].

The present study aims to characterize TauT in neural precursor cells obtained from the mesencephalon of mice embryonic brain, and cultured as neurospheres. Neurospheres are formed by aggregations of neural progenitor cells and may also contain multipotent stem cells. Altogether these cells are named 'neural precursor cells' (NPC). Under appropriate conditions, NPC differentiate into neurons, astrocytes and oligodendrocytes [Baizabal et al., 2003; Campos, 2004; Singec et al., 2006; Deleyrolle and Reynolds, 2009]. NPC have been extensively studied in the last years and some of their requirements for survival, proliferation, self-renewal and differentiation have been identified [Campos, 2004; Chaichana et al., 2006]. A recent study from our laboratory has shown an effect of taurine increasing proliferation of NPC isolated from mice embryonic mesencephalon and cultured as neurospheres [Hernández-Benítez et al., 2010]. The freshly excised tissue contains high levels of taurine characteristic of the embryonic mouse brain. The disaggregated cells are then cultured in the absence of fetal serum, which is the common source of taurine for cells in culture. Only NPC survive under these conditions, supported by specific growth factors [Tropepe et al., 1999]. After a few days in culture, NPC have lost all their taurine content, but addition of taurine to the culture medium fully replenishes the cell taurine pool [Hernández-Benítez et al., 2010]. This is indicative of an efficient mechanism of taurine accumulation, presumably via TauT. The present study aims to identify the presence and the functional expression of TauT in NPC.

Methods and Materials

Materials

DMEM/F_{12} with L-glutamine and 15 mM HEPES, Pen Strep (penicillin streptomycin), goat serum, human transferrin, human insulin, epidermal growth factor (EGF), fibroblast growth factor basic (bFGF), Glutamax 100X, and Alexa Fluor 568 (goat anti-rabbit) were from Gibco, Invitrogen. The antibody anti-rat C-tail TauT was from Alpha Diagnostics, Inc. Mouse nestin tail polyclonal antibody was purchased from Covance. The secondary antibody rabbit HRP-IgG was from Santa-Cruz Biotechnologies. Progesterone, sodium selenite, putrescine (1,4-diaminobutane-dihydrochloride), bisbenzimide H 33258 (Hoechst), D-(+)-glucose, Triton X-100, poly-L-lysine hydrobromide, Tris, ethylenediaminetetraacetic acid (EDTA), phenylmethylsulfonyl fluoride ($C_7H_7FO_2S$), bromophenol blue, NaCl, $MgSO_4$, KNO_3, choline chloride, sodium gluconate, and the amino acids β-alanine, hypotaurine, creatine, glycine and γ-aminobutyric acid (GABA) were from Sigma Aldrich. Albumin bovine serum (ABS) was from US Biological. Paraformaldehyde $(CH_2O)n$, chloride acid (HCl), KCl, KH_2PO_4 and $CaCl_2$ were from J.T. Baker, and HEPES from Roche. The acrylamide was from Ameresco, fat-free milk was from Svelty, Nestlé, and finally, taurine (ultra >99.5%) and ^3H-taurine were from Biochemika Fluka and American Radiolabeled Chemicals, respectively.

Cultures

Mesencephalon from CD1 13.5-day-old mouse embryos was dissected and transferred into ice-cold DMEM/F_{12} medium, triturated with a micropipette and the supernatant collected and centrifuged at 1,200 *g*/5 min. The pellet was resuspended and cells were plated in sterile 6-well plates (5×10^5 cells/well) with a medium containing Glutamax 100X, 25 μg/ml insulin, 100 μg/ml transferrin, 20 nM progesterone, 60 μM putrescine, 30 nM sodium selenite, 50 U/ml penicillin, 50 μg/ml streptomycin and supplemented with EGF plus bFGF (20 ng/ml each). Cultures were incubated at 37°C in humidified 5% CO_2/95% air atmosphere. After 5 days, primary neurospheres were collected by centrifugation (1,200 *g*/5 min), disaggregated, and the number of viable cells was determined by the trypan blue exclusion assay. For secondary neurospheres, primary neurospheres generated in EGF/bFGF condition were collected and centrifuged at 1,200 *g*/5 min, the pellet resuspended and neurospheres gently disaggregated. The dispersed cells were plated in sterile 6-well plates (2.5×10^5 cells/well) and formed secondary neurospheres, which were collected after 4 days for taurine uptake assays. Animals used in this research were acquired and cared for in accordance with the Norma Oficial Mexicana NOM-062-ZOO-1999.

Taurine Uptake Experiments

All experiments were carried out with NPC obtained from secondary neurosphere cultures. Secondary neurospheres were gently disaggregated, plated onto pretreated poly-L-lysine 24-well microplates (5×10^5 cells/well) with culture media and incubated at 37°C in humidified 5% CO_2/95 % air atmosphere during 2 h. Before each experiment, NPC were washed 3 times with medium containing (in mM): 135 NaCl, 5 KCl, 1.7 KH_2PO_4, 1.17 $MgSO_4$, 1 $CaCl_2$, 5 glucose and 10 HEPES (300 mosm, pH 7.4). Then cells were incubated for 30 min with ^{3}H-taurine (0.8 μCi/ml; 28 nM) under the condition indicated for each assay. At the end of the experiment, cells were washed and the accumulated radioactivity in cells was measured in a liquid scintillation counter. Taurine uptake is calculated as nmol/mg protein/min. For assays on the Na^+/Cl^- dependence of taurine uptake, NaCl in the medium was replaced by equiosmolar concentrations of choline chloride (for Na^+ dependence) or sodium gluconate (for Cl^- dependence). KNO_3 substituted NaCl in experiments to quantitate the saturable and non-saturable components of ^{3}H-taurine. Osmolarity of all media was verified in a freezing point osmometer from Precision Systems Inc. (Natick, Mass., USA).

SDS-PAGE and Western Blotting

Neurospheres were collected, washed in PBS and homogenized in a buffer containing 10 mM of Tris-HCl (pH 7.4), 1 mM EDTA and 0.5 mM $C_7H_7FO_2S$. Samples were then sonicated for 20 s, the homogenized cells centrifuged at 1,000 *g* for 10 min, and the supernatant collected and stored at 0°C until processing. The protein content of samples was in the range of 30–55 μg and was measured by the Bradford assay. Equal amounts of protein were separated by polyacrylamide gel electrophoresis (10%), followed by transfer to a 0.2-μm nitrocellulose membrane (Trans-Blot, Bio Rad, USA). The membrane was blocked with a solution of TBS + fat-reduced milk (5%) + ABS (1%) during 1 h. Proteins of interest were then detected using appropriate antibodies: anti-rat TauT (1:750) incubated overnight, and secondary antibody rabbit HRP-IgG (1:2,000) incubated for 1 h. The band reactivity was identified using a chemiluminescence detection kit ECL-Plus (Amersham GE Healthcare, UK).

Immunocytochemistry

The neurospheres obtained from secondary cultures were gently disaggregated, washed and plated onto pretreated poly-L-lysine 96-well microplates (for epifluorescence) or coverslips (for confocal microscopy), with culture medium, and samples were incubated at 37°C in humidified 5% CO_2/95% air atmosphere during 2 h. Then, cells were fixed in 4% paraformaldehyde (15 min), washed (3 times, 5 min each) with PBS/ABS 0.1%, permeabilized/blocked with PBS/ABS 0.1% + 10% goat serum + 0.3% Triton X-100, during 1 h at room temperature. Nestin- and TauT-positive cells were identified by 24-hour incubation with anti-mouse nestin tail (1:200) or anti-rat C-tail TauT (1:100) primary antibodies, followed by 1-hour incubation with Alexa Fluor 568 secondary antibody (1:200). Nuclei were counterstained with Hoechst (1:500). Microphotographs were visualized in an epifluorescence microscope Olympus IX71 with a 20× objective and analyzed by an Image-Pro plus 6.0 software. Subcellular distribution of TauT reactivity was examined by confocal microscopy. Optical slices in the z-axis were taken at 0.75-μm intervals. The samples were scanned on a confocal laser scanning microscope FV1000, with a Plan-Apo 60X NA 1.45 objective. Images were processed with an Olympus FV10-ASW 2.1 software.

Data Analysis

Statistical differences between experimental groups were determined by ANOVA followed by Tukey's test. Statistically significant differences were considered at * $p < 0.01$. All data are given as means ± SEM. For image processing, microphotographs were analyzed using Adobe Image Ready 7.0®, Image-Pro plus 6.0 software® and Olympus Fluoview FV10-ASW 2.1®.

Results

Culture and Characterization of NPC

Cell cultures obtained from the mesencephalon of 13.5-day-old mouse embryos were maintained in a growth-promoting medium containing EGF plus bFGF (20 ng/ml each). Free-floating neurospheres were formed in cultures over the course of 5 days, and thereafter, cells from dissociated neurospheres were cultured to generate other neurospheres (secondary neurospheres). In accordance with our previous study [Hernández-Benítez et al., 2010], essentially all the neurosphere-forming cells (97.4 ± 1.1%) were positive to nestin, which is a marker for NPC. Cells obtained from secondary neurosphere disaggregation were plated for ^{3}H-taurine uptake experiments.

Functional Characterization of ^{3}H-Taurine Transport in NPC

The time course of taurine uptake by NPC measured as ^{3}H-taurine accumulation was linear within the first 30 min (results not shown). Therefore, all subsequent experiments were performed at this time. For kinetic analysis of taurine uptake, NPC were incubated with increasing

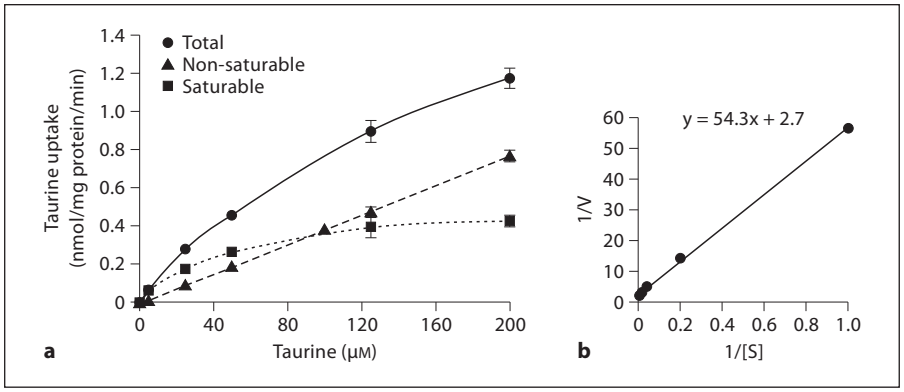

Fig. 1. Kinetic analysis of ^{3}H-taurine uptake by NPC. Cells were incubated during 30 min with ^{3}H-taurine (0.8 μCi/ml, 28 nM) and unlabeled taurine over a concentration range of 1–200 μM. **a** ● = Total taurine uptake; ▲ = uptake measured in the absence of NaCl, replaced by KNO_3 (non-saturable component); ■ = uptake resulting from subtracting the Na^+/Cl^--independent uptake (saturable component). Uptake rates were calculated from the amount of radioactivity in the cells after the uptake period and the unlabeled taurine in the incubation media. **b** Lineweaver-Burk plot of the saturable component. The V_{max} and K_m constants were calculated using the Lineweaver-Burk equation. Results are means ± SE of 6–20 experiments. V = Taurine uptake; S = taurine concentration.

concentrations of unlabeled taurine, in the range of 1–200 μM and 0.8 μCi/ml of ^{3}H-taurine (28 nM). Accumulation of ^{3}H-taurine was concentration dependent up to 200 μM (fig. 1a). Total taurine uptake could be separated in a non-saturable and a saturable component. The non-saturable component was identified by measuring ^{3}H-taurine uptake in a medium with equiosmolar substitution of NaCl by KNO_3. Taurine accumulated under this condition was subtracted from the total ^{3}H-taurine uptake. This procedure evidenced the saturable component (fig. 1a). The kinetic constants of this saturable component were calculated using the Lineweaver-Burk equation. The Lineweaver-Burk plot (fig. 2b) revealed a V_{max} of 0.39 ± 0.04 nmol/mg protein/min and a K_m of 21.7 ± 2.6 μM.

Taurine uptake by NPC was Na^+ and Cl^- dependent. Figure 2a shows ^{3}H-taurine uptake measured in the presence of increasing Na^+ concentrations (0–135 mM) and a fixed concentration of Cl^-, made by equiosmolar replacement of NaCl by choline chloride. Figure 2b shows ^{3}H-taurine uptake measured in the presence of increasing concentrations of Cl^- (0–135 mM) and a fixed concentration of Na^+, made by equiosmolar replacement of NaCl by sodium gluconate. Results showed a clear Na^+/Cl^- dependence of taurine uptake in NPC. The concentrations of Na^+ and Cl^- at which half-maximal ^{3}H-taurine transport occurs were 95.2 ± 9 and 15.2 ± 2.9 mM, respectively. The ^{3}H-taurine transport was a sigmoidal function of the concentration of Na^+, with a Hill coefficient of 2.032 (fig. 2a). The ^{3}H-taurine transport was a hyperbolic function of the concentration of Cl^-, and the Hill coefficient was 1.183 (fig. 2b). These results indicate an apparent Na^+/Cl^-/taurine stoichiometry of 2:1:1.

The specificity of the saturable ^{3}H-taurine uptake in NPC was assessed by measuring the competitive effect of the β- and γ-amino acids, hypotaurine, β-alanine and GABA, all tested at 1-mM concentration. Creatine was used as a control. Figure 3 shows that the most potent blocker was hypotaurine, which reduced the ^{3}H-taurine uptake by 92%. β-Alanine and GABA inhibited ^{3}H-taurine uptake by 77 and 69%, respectively, whereas creatine had no significant effect (fig. 3).

TauT Protein Expression and Subcellular Localization

TauT protein in NPC was revealed by Western blotting and immunocytochemistry, using an anti-rat C-tail TauT antibody. TauT was detected in NPC as a single band of about 70 kDa. Figure 4 shows the TauT band from lysates of NPC and tissue (embryonic mesencephalon). TauT immunoreactivity in cells forming neurospheres was visualized by epifluorescence microscopy (fig. 5a, b). Counting the number of TauT-positive neurosphere-forming cells revealed that 98.8% (± 1.3) expressed the transporter. The subcellular distribution of the TauT immunoreactivity in NPC from disaggregated neurospheres was examined by confocal microscopy. NPC are small cells (12.9 ± 0.6 μm), with large nuclei (9.1 ± 0.4 μm) (fig. 5c, d). TauT immunoreactivity was predominantly localized at the cell membrane. Some TauT reactivity was also located at the cytosol. Very little reactivity was found at the nucleus (fig. 5d).

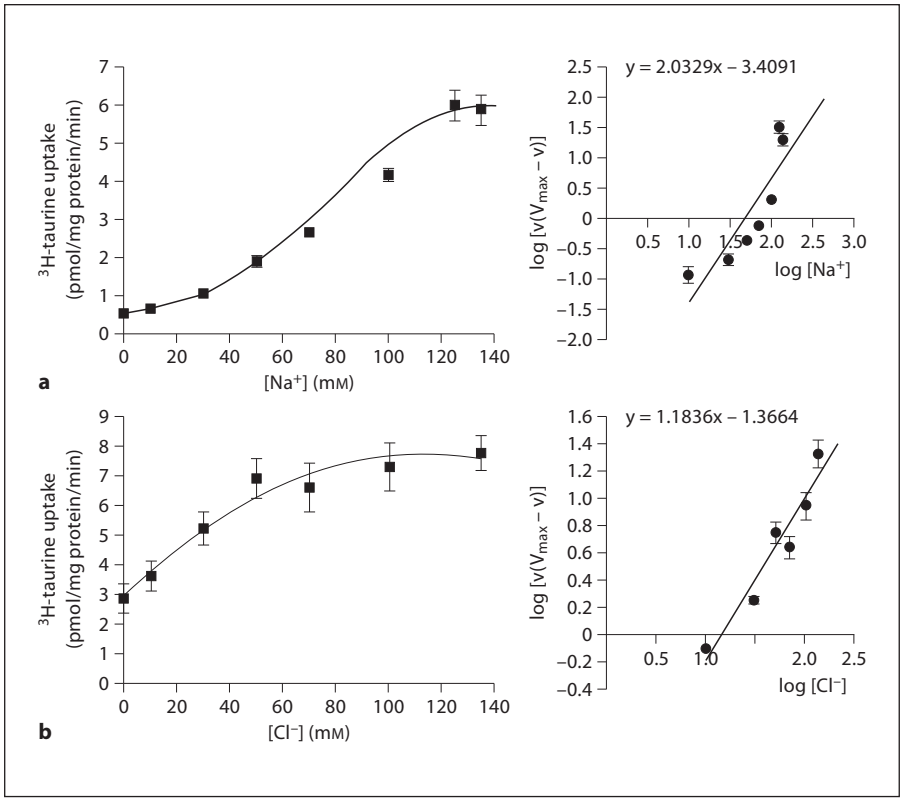

Fig. 2. Na^+ and Cl^- dependence of taurine uptake in NPC. **a** Uptake of ^3H-taurine measured in the presence of increasing Na^+ concentrations (0–135 mM) and a fixed concentration of Cl^- made by equiosmolar replacement of NaCl by choline chloride. **b** Uptake of ^3H-taurine measured in the presence of increasing concentrations of Cl^- (0–135 mM) and a fixed concentration of Na^+, made by equiosmolar replacement of NaCl by sodium gluconate. The Hill plots are from of the corresponding data in **a** and **b**. Results are means ± SE of 6 experiments.

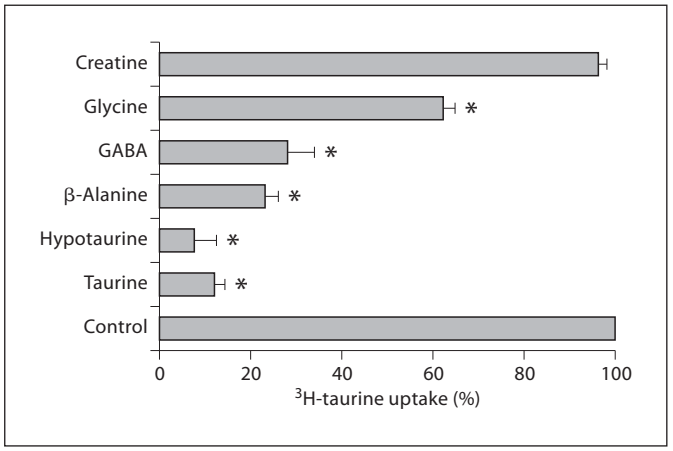

Fig. 3. Substrate specificity of taurine uptake in NPC. Na^+/Cl^--dependent ^3H-taurine uptake was measured as described in figure 2a, in the absence (control) or presence of unlabeled taurine, hypotaurine, β-alanine, GABA, glycine or creatine, all 1 mM. Bars represent the percentage reduction in ^3H-taurine uptake when measured in the presence of unlabeled amino acids. Results are means ± SE of 12 experiments. * $p < 0.01$.

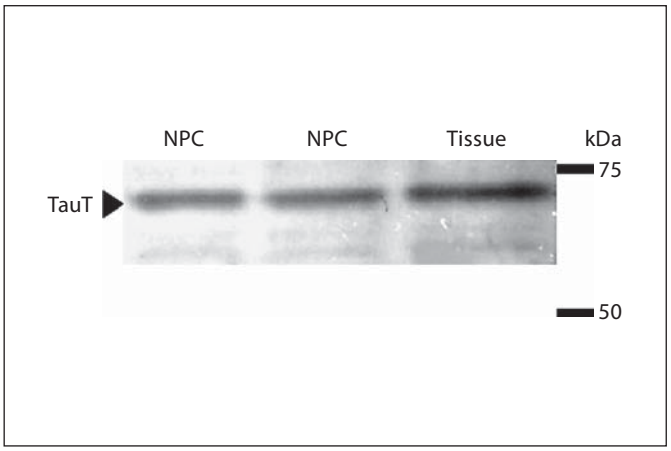

Fig. 4. TauT protein expression in NPC and the mesencephalon tissue revealed by immunoblotting. Proteins from lysates of the cells or tissue were separated by SDS-PAGE (10%) and TauT was detected using an anti-rat C-tail TauT antibody (see Methods). Representative assay from 6 separate experiments.

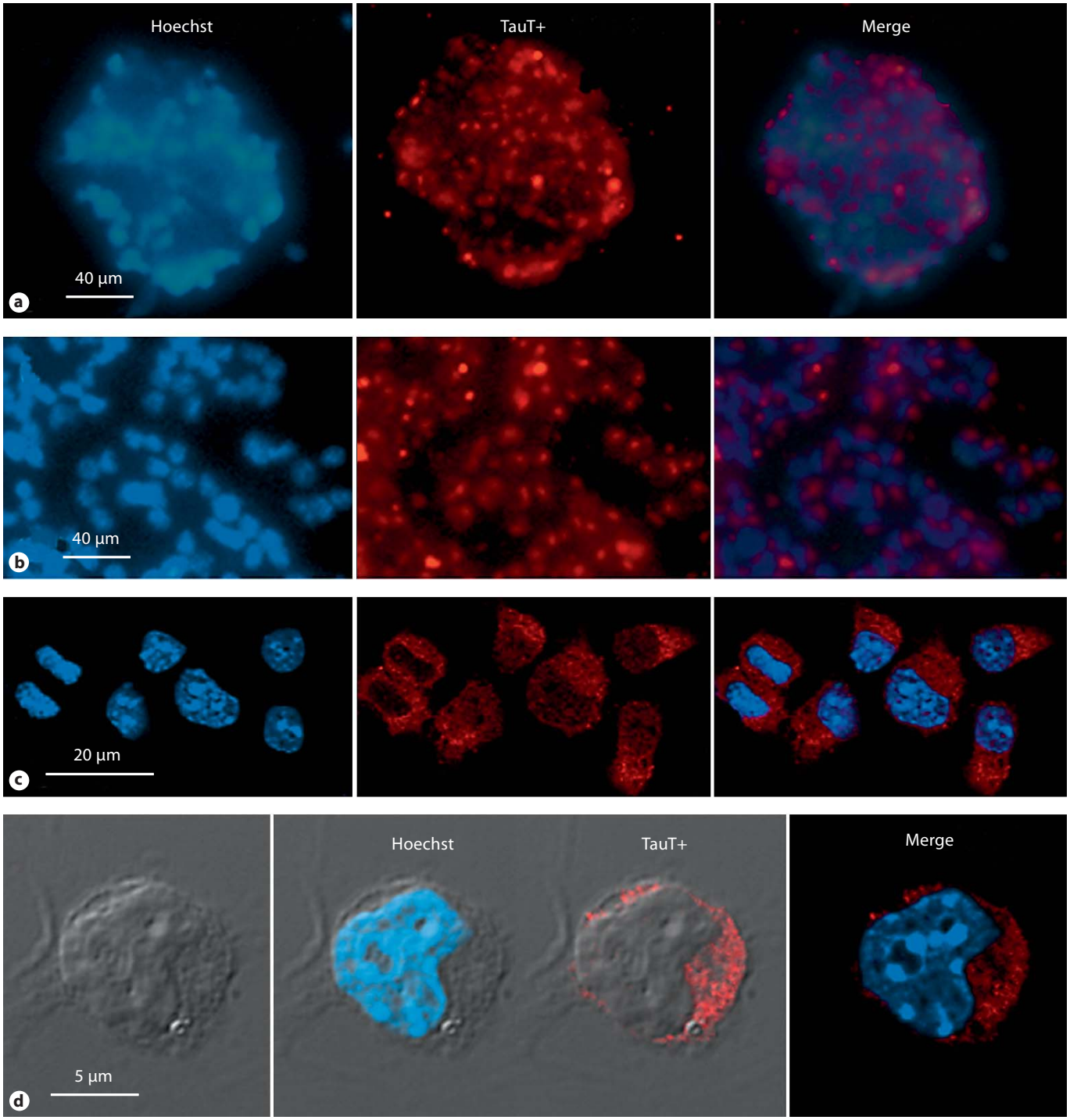

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Fig. 5. Identification and localization of TauT in NPC. TauT reactivity detected by immunocytochemistry, using a primary antibody anti-TauT, followed by the secondary antibody Alexa Fluor 568. Nuclei were counterstained with Hoechst. **a**, **b** TauT reactivity in neurospheres (**a**) or in disaggregated cells (**b**) visualized by epifluorescence microscopy. **c** TauT reactivity in NPC, visualized by confocal microscopy (see Methods). **d** Subcellular localization of TauT reactivity by confocal microscopy in an optical slice taken in the z-axis at 0.75 µM intervals. Left column: a differential interference contrast Nomarski image of a single NPC; middle column: cell image with Hoechst-stained nucleus and TauT reactivity; right column: merge (Hoechst and TauT). The photomicrographs are from a representative assay.

Discussion

The present study provides evidence of the functional expression of TauT in NPC obtained from the mice embryonic mesencephalon. To our knowledge, this is the first detailed report on the expression and functional characterization of TauT in NPC. Essentially all the NPC cells were TauT positive, regardless of their growing state or differentiation commitment, stressing the importance of this mechanism to maintain taurine levels in NPC.

Taurine transport is an essential mechanism to maintain taurine levels in brain cells, particularly in the embryonic brain, where the contribution of endogenous synthesis seems only marginal or absent. No evidence of mRNA for cysteine dioxygenase or cysteine sulfinate decarboxylase is found in glia or neurons of the developing rodent brain [Rassin et al., 1981; Fujita et al., 2006]. Only in differentiated astrocytes, some cysteine sulfinate decarboxylase activity has been detected [Reymond et al., 1996]. Our previous study on the effect of taurine on NPC proliferation, showing a rapid depletion of the taurine pool in NPC grown in the absence of taurine, suggests that these cells also lack the machinery for endogenous biosynthesis [Hernández-Benítez et al., 2010]. The present results showing an efficient mechanism for taurine transport in NPC, explain the fact that NPC cultures grown in the presence of taurine rapidly recover the high taurine levels found in the embryonic tissue. TauT is expressed in a large variety of cells and tissues, in accordance with the ubiquitous presence of taurine in animal cells. TauT has been cloned from a variety of tissues including the rat and mouse brain [Liu et al., 1992; Smith et al., 1992]. TauT cDNA encodes for a protein of about 70 kDa, with high homology between tissues and species. The functional characterization of taurine transport also shows remarkable similarities between different cell types, including the NPC examined in the present study.

Taurine uptake in NPC showed a saturable, Na^+/Cl^--dependent component and a non-saturable, Na^+/Cl^--independent component. The K_m and V_{max} values of the saturable uptake component were similar to those found in brain cells. The V_{max} of 0.39 nmol/mg protein/min found in NPC was within the range reported for cerebellar granule cells (0.64), astrocytes (0.2), mouse developing neurons (0.67) and for the rat brain-cloned TauT (0.4) [Sánchez-Olea et al., 1991; Schousboe et al., 1991; Smith et al., 1992; Fujita et al., 2006]. Similarly, the K_m value of 21.7 μM found in NPC was of the same order of magnitude as the K_m of 10.6 μM in mouse cerebrocortical neurons, 4.5 μM in mouse brain, 35 μM in mouse astrocytes and 43 μM in TauT cloned from rat brain [Sánchez-Olea et al., 1991; Liu et al., 1992; Smith et al., 1992; Fujita et al., 2006]. TauT in NPC shows an apparent stoichiometry of 2:1:1 for Na^+/Cl^-/taurine transport. Since at physiological pH taurine is a zwitterion without net charge, the TauT operation is electrogenic. TauT in NPC was specific for the β-amino acids, taurine, hypotaurine and β-alanine, with some interaction also with the γ-amino acid GABA. These features of TauT in NPC are similar to those reported for TauT in other brain and non-brain cells [Smith et al., 1992; Voss et al., 2004; Fujita et al., 2006].

TauT protein expression in NPC identified by Western blotting analysis showed the 70-kDa protein, as has been reported in all cell types examined so far. TauT in NPC was found localized at the plasma membrane and some at the cytosol, but it was essentially absent from the nuclear membrane. In NIH3T3 fibroblasts, TauT seems to be inside the nuclear compartment [Voss et al., 2004]. So far, no other studies reported TauT subcellular distribution. More information is necessary to clarify whether the nuclear location of TauT is cell specific.

TauT has a critical function in the well-established role for taurine as osmolyte in a large variety of cells, including brain cells. Reductions in external osmolarity evoke an immediate efflux of taurine, of a magnitude proportional to the hypotonic stimulus. This taurine mobilization occurs through a volume-sensitive taurine leak pathway. In brain cells, the non-saturable component of taurine uptake is highly increased by hyposmolarity and presumably represents the volume-sensitive taurine leak pathway [Sánchez-Olea et al., 1991; Schousboe et al., 1991]. Similarly, the non-saturable component of NPC was increased by hyposmolarity (not shown). Taurine efflux through this pathway, together with the efflux of other organic osmolytes, contributes to reestablish the osmotic equilibrium. When hypotonicity is large or persistent, the cells become taurine depleted. Upon osmolarity correction, the cell taurine pool is replenished by the TauT operation. The transporter also plays a key role in the cell adaptive response to hypertonicity. TauT upregulation via the TonEBP pathway is a characteristic cell response to a hypertonic condition, directed to increase cell taurine concentration, and together with other osmolytes, counteracts cell volume loss [Ito et al., 2004]. The remarkable similarities of the distinctive features of TauT in a variety of cells, tissues and organs stress the importance of this universal mechanism for cell homeostasis.

A previous study from our laboratory showed an effect of taurine increasing the number of NPC in cultures ob-

tained from mice mesencephalon. Cells cultured in taurine-free conditions have a negligible taurine cell content, while those grown in the presence of taurine reach the high taurine levels characteristic of the developing brain. The mechanisms of taurine action increasing NPC proliferation are still unknown. The present demonstration of an ubiquitous and active taurine transporter in NPC suggests that taurine action may be exerted from outside as well as from inside the cell.

Embryonic NPC have been used in transplantation experiments into the adult brain. To optimize this procedure, cells may require the presence of cues or factors found in the embryonic brain milieu but absent in the adult. Taurine may be one of these factors, and the functional TauT in NPC cells will provide the mechanism for increasing cell taurine levels in the transplanted embryonic cells.

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