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Multiple mechanisms mediate the taurine-induced proliferation of neural stem/progenitor cells from the subventricular zone of the adult mouse

Gerardo Ramos-Mandujano [1], Reyna Hernández-Benítez [1,*], Herminia Pasantes-Morales [**]

División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico

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Abstract Taurine was previously reported to increase the proliferation of neural precursor cells (NPCs) from subventricular zone of the mouse brain. The results of a study that aimed to understand the mechanisms of this effect are presented here. Because taurine was not found in NPC nuclei, direct interactions with nuclear elements seem unlikely. A gene expression profile analysis indicated that genes that are regulated by taurine have roles in i) proliferation, including the Shh and Wnt pathways; ii) cellular adhesion; iii) cell survival; and iv) mitochondrial functioning. Cell cycle analysis of propidium iodide and CFSE-labeled cells using flow cytometry revealed an increase in the number of cells in the S-phase and a decrease in those in the G0/G1 phase in taurine-treated cultures. No changes in the length of the cell cycle were observed. Quantification of the viable, apoptotic, and necrotic cells in cultures using flow cytometry and calcein-AM, annexin-V, and propidium iodide staining showed reductions in the number of apoptotic and necrotic cells (18% to 11% and 13% to 10%, respectively) and increases in the number of viable cells (61% to 69%) in the taurine-treated cultures. Examination of the relative mitochondrial potential values by flow cytometry and rhodamine123 or JC-1 staining showed a 44% increase in the number of cells with higher mitochondrial potential and a 38% increase in the mitochondrial membrane potential in taurine cultures compared with those of controls. Taken together, the results suggest that taurine provides more favorable conditions for cell proliferation by improving mitochondrial functioning.

Abbreviations: NPCs, neural precursor cells; SVZ, subventricular zone; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; BrdU, 5-bromo-2′-deoxyuridine; Shh, sonic hedgehog; Wnt, wingless integrated; CFSE, carboxyfluorescein diacetate succinimidyl ester; $\Delta\psi m$, mitochondrial membrane potential; Rh123, rhodamine 123; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi-dazolylcarbocyanine iodide; mtRNA, mitochondrial transfer RNA; GEO, Gene Expression Omnibus; qPCR, quantitative real-time polymerase chain reaction.

* Correspondence to: R. Hernández-Benítez, División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad Universitaria, Circuito Exterior, 04510 Mexico DF, Mexico.
** Correspondence to: H. Pasantes-Morales, División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad Universitaria, Circuito Exterior, 04510 Mexico DF, Mexico. Fax: +52 5622 5607.
E-mail addresses: reynah@email.ifc.unam.mx (R. Hernández-Benítez), hpasante@ifc.unam.mx (H. Pasantes-Morales).
[1] These authors contributed equally to this study.

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Introduction

Taurine (2-aminoethane sulfonic acid) is present in most animal cell types. This sulfur β-amino acid is often at high concentrations (in the mM range), particularly in excitable tissues. Taurine is mostly found soluble in the cytosol. It is not a protein constituent, and it participates in a small number of cell metabolic reactions, the most important of which is the synthesis of taurocholic acid, a major constituent of bile (Huxtable, 1992). In most tissues, the taurine pool is maintained through dietary intake and/or synthesis in the liver. The transfer of taurine to cells and tissues is performed by an energy Na^+/Cl^--dependent transporter (TauT) specific for β-amino acids, which is functionally expressed in most cells (Tappaz, 2004). Taurine participates as a compatible osmolyte in cell volume regulation in a wide variety of cells, including brain cells (Pasantes-Morales et al., 2000). Taurine also shows protective effects in cells challenged by conditions involving oxidative stress (Schaffer et al., 2009). Recent evidence related taurine with mitochondrial tRNA post-transcriptional modification (Schaffer et al., 2009; Suzuki et al., 2002).

Taurine is important for optimal brain development. Its concentration in the developing brain is 3–4-times higher than that in the adult brain, a pattern consistently found in most species regardless of the differences in their taurine concentrations (Sturman, 1993). Substantial support of taurine requirement for brain development came from studies of taurine-deficient animals. In taurine-deficient newborn cats, the maturation and migration of cerebellar granule neurons are impaired. The ontogeny of neuroblasts and glioblasts in the visual cortex is delayed and the layer organization is defective. In the spinal cord, the alignment of the dorsal root nerves is disturbed (Sturman, 1992). Similarly, monkeys fed formulas lacking taurine exhibited defective organization of the cortical layers in the visual cortex (Neuringer et al., 1990). Recent studies have shown effects of taurine increasing the proliferation rate of stem/progenitor cells obtained from the embryonic mouse mesencephalon (Hernandez-Benitez et al., 2010), the hippocampus dentate gyrus (Shivaraj et al., 2012) and the human fetal brain (Hernandez-Benitez et al., 2013). These effects of taurine were also found in NPCs from the SVZ of the lateral ventricles of adult mice (Hernandez-Benitez et al., 2012). Treatment with taurine in human fetal NPCs and NPCs from the SVZ increased the proportion of neurons formed during the differentiation process. The mechanisms of these actions of taurine are poorly understood. Understanding of these mechanisms was addressed in the present study, which was performed using NPCs obtained from the SVZ. Considering that taurine may act via different mechanisms, we conducted a DNA microarray analysis, which showed that taurine evoked changes in the transcription of genes involved in proliferation, mitochondrial functioning and cell adhesion. The transcription of cell survival genes also changed in the presence of taurine.

Materials and methods

Culture of neural precursors cells from the subventricular zone

Neural precursor cells were obtained from the subventricular zone of adult mouse brains and cultured as neurospheres (Rietze and Reynolds, 2006). Tissue from the subventricular zones of adult CD1 mice was mechanically dissociated to yield a cell suspension. The cells were incubated in medium containing DMEM/F12, 1 × glutamax, 25 μg/mL insulin, 100 μg/mL transferrin, 20 nM progesterone, 60 μM putresine, 30 nM sodium selenite, 50 U/mL penicillin, 50 μg/mL streptomycin, and the growth factors EGF and FGF2 (20 ng/mL each) in sterile 6-well plates (30×10^3 cells/well). To obtain taurine cultures, the medium was supplemented with 10 mM taurine (Fluka BioChemica, Sigma-Aldrich). The cultures were maintained at 37 °C in humidified 5% CO_2/95% air atmosphere for 9–15 days. The neurospheres that had formed were mechanically disaggregated, and the cells were seeded as secondary cultures. The animals used in this research were utilized and cared for in accordance with the Norma Oficial Mexicana NOM-062-ZOO-1999.

Immunocytochemical detection

For BrdU incorporation, the cells with a (10 μM) BrdU pulse (1.5 h) were collected, washed, resuspended in culture medium, and plated onto 96-well microplates that were pre-treated with poly-L-lysine. The cells were fixed using 4% paraformaldehyde for 15 min, washed with PBS/0.1% BSA, and incubated with PBS-2 N HCl solution (37 °C, 15 min). Then, they were permeabilized/blocked using PBS/0.1% BSA + 10% GS (goat serum) + 0.3% Triton 100× (1 h at room temperature), incubated overnight at 4 °C with rat anti-BrdU antibody [1:100] (Accurate Chemical OBT0030), and then incubated (1 h) with Alexa Fluor 488-conjugated goat anti-rat IgG [1:200] (Molecular Probes, Life Technologies A11006). The nuclei were counterstained using 2 μg/mL of Hoechst 33258 (Sigma-Aldrich). Images were obtained using an epifluorescence Olympus IX71 microscope with a 10× objective and analyzed using ImageJ software. To evaluate the cell localization of taurine, neurospheres from control or taurine cultures were disaggregated and NPCs plated as a monolayer on coverslips were pretreated with poly-L-lysine. The NPCs were fixed using a glutaraldehyde (1%)/paraformaldehyde (4%) solution, permeabilized/blocked, incubated overnight at 4 °C with a rabbit anti-taurine-specific antibody [1:200] (Abcam, ab9448), and then incubated (1 h) with Alexa Fluor 568-conjugated goat anti-rabbit IgG [1:200] (Molecular Probes, Life Technologies A11011). The nuclei were counterstained using Hoechst. The cellular localization of taurine reactivity was determined by confocal microscopy with optical slices in the *Z* axis taken at 0.75 μm intervals. The samples were examined using a confocal laser scanning FV1000 microscope with a Plan-Apo 60× NA 1.45 objective, and the images were processed using Olympus FV10-ASW 2.1 software.

Microarray processing

The total RNA from three NPC control cultures and three NPC taurine cultures was purified using the RNeasy mini kit (Qiagen). The RNA (10 μg) was used to produce tagged complementary DNA (cDNA) probes incorporating dUTP-Alexa555 or dUTP-Alexa647 employing the Firs-Strand cDNA labeling kit (Invitrogen). The mouse 65-mer oligo library