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Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells*

Sissy M. Jhiang, Linda Fithian, Patricia Smanik, Jeffrey McGill, Qiang Tong and Ernest L. Mazzaferri

Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, USA

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A cDNA clone encoding a taurine transporter, designated HTAU, has been isolated from human thyroid. It contains an open reading frame encoding a protein of 619 amino acids with a calculated molecular weight of 69,675 Da. The predicted amino acid sequence of HTAU is highly homologous to those of dog kidney and rat brain. The HTAU mRNA was detectable in various human tissues examined. Transient expression of HTAU in COS-7 cells conferred a higher taurine uptake. The taurine uptake in FRTL-5 cells appears to be regulated by thyrotropin through cAMP. Finally, a higher taurine uptake may be associated with a higher proliferation rate in some cultured cell lines.

Human; Thyroid; Taurine; Transporter; cDNA; Sequence

1. INTRODUCTION

Taurine, 2-aminoethanesulfonic acid, is a major intracellular β-amino acid in mammals. It is most abundant in tissues that are excitable, are rich in membranes, and that generate oxidants [1]. Proposed functions of taurine include: membrane stabilization, detoxification, antioxidation, osmoregulation, modulation of calcium flux, and a role as a neurotransmitter or neuromodulator. It is well established that taurine is essential for retinal integrity and function [2]. Furthermore, taurine deficiency has been reported in several members of a family who had mild depression, insomnia, anorexia, dysphagia, dyspnea, and loss of visual depth perception [3]. Recently, taurine has been shown to have a protective effect on myocardial alterations caused by prolonged treatment with norepinephrine in rats [4]. Significant treatment effect was observed on systolic left ventricular function in taurine-treated patients with congestive heart failure [5]. It is generally accepted that taurine has inhibitory neurotransmitter properties in the retina and central nervous system. Recently, it has been suggested that taurine may modulate neuroactivity by inhibiting the phosphorylation of specific proteins regulated by the signal transduction system in the brain [6]. To date, it is still unknown how a single molecule, taurine, possesses such diversified function in various tissues of animals. Furthermore, little is known about the molecular properties, regulation and expression of the taurine transporter.

In an attempt to clone Na^+-dependent transporters from the human thyroid gland, we have isolated a cDNA clone encoding the human taurine transporter. The primary structure of the human taurine transporter was determined, and compared with the recently cloned dog kidney [7] and rat brain taurine transporters [8]. Additionally, the distribution of taurine transporter in various human tissues was demonstrated. The regulation of taurine uptake in thyroid cells was investigated. Finally, taurine uptake was measured in several cultured cell lines.

Correspondence address: S.M. Jhiang, 446 McCampbell, 1581 Dodd Drive, The Ohio State University, Columbus, OH 43210, USA. Fax: (1) (614) 292-1550.

* The cDNA sequence of HTAU has been deposited in EMBL.

2. MATERIALS AND METHODS

2.1. *Cloning and sequencing*

A human thyroid cDNA library, cloned into the *Eco*RI site of the lambda gt10 cloning vector, was obtained from Clontech laboratory (Palo Alto, CA). The cDNA library was screened with ^{32}P-labeled DNA fragments, which include the DNA sequence encoding the transmembrane regions I to V of the GABA neurotransmitter [9]. The hybridized filters were washed at low stringency (2 × SSC, 0.1% SDS, 50°C). One of the positive clones containing an insert of approximately 4 kb, designated HTAU, was further characterized and sequenced using the USB sequenase kit. The DNA sequence was then subjected to a homology search using the BLAST network service provided by NCBI [10].

2.2. *Tissue distribution of HTAU*

Reverse transcription-polymerase chain reaction (RT-PCR) was performed to identify tissues expressing HTAU mRNA. Two oligomers derived from amino acids 86–92 and 309–315 of HTAU were used for the PCR reaction. Therefore, a target fragment of 687 bp was expected. The PCR products were separated by gel electrophoresis and blotted to nylon membrane. The ^{32}P-labelled DNA fragments of HTAU were used as probes to confirm the HTAU-specific PCR products.

2.3. *Functional expression of HTAU in COS-7 cells*

The HTAU cDNA was subcloned into the eukaryotic expression vector pSG5 (Stratagene). The orientation of the cDNA was determined by restriction analysis. The HTAU was introduced into COS-7 cells, a monkey kidney cell line (ATCC CRL 1651), by Lipofectin (BRL). Taurine or iodide uptake in these transiently transfected cells was measured 2 days after the transfection. For the taurine uptake assay, cells were incubated with 70 nM [1,2-^{3}H]taurine (28 Ci/mmol) in fresh medium for 1 h at room temperature, followed by washing 3 times with ice-cold Hanks Balanced Salt Solution (HBSS). The cells were then solubilized with 1% SDS for [^{3}H]taurine counting by liquid scintillation. For the iodide uptake assay [11], cells were incubated in 500 μl of fresh medium containing 1 μCi of $Na^{125}I$ and 5 μM NaI for 40 min at 37°C. Cells were then washed once with HBSS, and incu-

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                                    ◆                ■◆         I
Hum      MATKEKLQCLKDFHKDMVKPSPGKSPGTRPEDEAEGKPPQREKWSSKIDFVLSVAGGFVG   60
Dog      ................IL..........................................   60
Rat      ................IL................D.........................   60

                                    II                     ◆
Hum      LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEIIIGQYTSEGGITCWEKICPLF   120
Dog      .............................G........V.....................   120
Rat      ......................................V.....................   120

                  III                              *           ■   *
Hum      SGIGYASVVIVSLLNVYYIVILAWATYYLFQSFQKELPWAHCNHSWNTPHCMEDTMRKNK   180
Dog      .......I.......I..VI..............S..............Q..........   180
Rat      .......I...........................D.............Q.....L.R.E   180

                  *                        ■            IV
Hum      SVWITISSTNFTSPVIEFWERNVLSLSPGIDHPGSLKWDLALCLLLVWLVCFFCICKGVR   240
Dog      .L...L.TK......T...........S...D.......................W...K   240
Rat      .H.VSL.AA..................S...........................W....   240

          ■              V                         ◆
Hum      STGKVVYFTATFPFAMLLVLLVRGLTLPGAGRGIKFYLYPDITRLEDPQVWIDAGTQIFF   300
Dog      ...............................A..........S.................   300
Rat      ...............................E........N.S.................   300

                              ■
          VI                  ◆               VII
Hum      SYAICLGAMTSLGSYNKYKYNSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVDIA   360
Dog      ............................................................   360
Rat      ............................................................   360

                                    VIII                     ◆
Hum      DVAESGPGLAFIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPS   420
Dog      ..................................................V.........   420
Rat      ............................................................   420

                         IX                                  X
Hum      FLRKGYRREIFIAFVCSISYLLGLTMVTEGGMYVFQLFDYYAASGVCLLWVAFFECFVIA   480
Dog      .....F........M.........S...................................   480
Rat      .............I..............................................   480

                                       XI                    *
Hum      WIYGGDNLYDGIEDMIGYRPGPWMKYSW-VITPVLCVGCFIFSLVKYVPLTYNKTYVSPT   539
Dog      ....S.......................A.V.......................V..Y..   540
Rat      ............................A....A....................V.RY.D   540

                                                 ■
                       XII                       ◆
Hum      WAIGLGWSLALSSMLCVPLVIVIRLCQTEGPFLVRVKYLLTPREPNRWAVEREGATPYNS   599
Dog      ..............M.....M..............L....................LQLP   600
Rat      .......G......V.I......L..R....LR..I...I.................FH.   600

Hum      R-TVMNGALVKPTHIIVETMM                                          619
Dog      PGRERGSHETD.H.SRDHDVSSWADGPLPCCLLTLDSHRTRFTELSICTRIVFFF        655
Rat      .A.L.....M..S.V......                                          621
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Fig. 1. Alignment of the human, dog, and rat taurine transporters. The 12 putative transmembrane domains (I–XII) are underlined. (.) indicates identical amino acids. The potential N-linked glycosylation sites (*), protein kinase C phosphorylation sites (■), and casein kinase II phosphorylation sites (◆) are indicated.

bated in 500 μl of 95% ethanol for 20 min at room temperature. The ethanol was then collected and the ^{125}I content was measured by gamma-counting. Both taurine- and iodide-uptake activities were expressed as cpm × 1,000/10^5 cells.

2.4. *TSH regulation of taurine uptake in FRTL-5 cells*

FRTL-5 cells, established from normal rat thyroid cells (ATCC CRL 8305), conserve most of the physiological characteristics of the thyroid, such as thyrotropin (TSH)-dependent iodide uptake and growth. These cells were normally maintained in Coon's F12 medium containing six hormones and 5% fetal calf serum (FCS) [12]. In this study, FRTL-5 cells were deprived of TSH for 8 days, followed by 8-bromo-cAMP, or TSH treatment for 3 days before taurine- or iodide-uptake assay was performed.

2.5. *Proliferation rate of human thyroid carcinoma derived cell lines*

TPC-1 and NPA are cells derived from human papillary thyroid carcinomas. TT (ATCC CRL 1803) and WRO cells were established from human medullary thyroid carcinoma, and follicular thyroid carcinoma, respectively. TPC-1 cells were maintained in DMEM high glucose medium containing 5% FCS. NPA, WRO, and TT cells were maintained in RPMI-1640 medium containing 10% FCS. To determine the proliferation rate of these cells, 5×10^4 cells per well were plated on 24-well plate, and cells were harvested for cell number counting after 21, 46.5 and 69.5 h incubation. Taurine uptake was measured after cells were plated for 21 h.

2.6. *Statistical analyses*

ANOVA was used to compare the results among three or more groups with Newman–Keuls test for multiple comparison. A P-value equal or less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. *The primary structure of HTAU*

The 3,957-nucleotide cDNA contains an open reading frame composed of nucleotides 14–1,873. This open reading frame encodes a protein of 619 amino acids with a calculated molecular weight of 69,675 Da. The amino acid sequence encoded by HTAU (Fig. 1) shows 91% identity with the dog kidney taurine transporter [7], and 93% identity with the rat brain taurine transporter [8]. Most of the non-homologous residues are located in the second extracellular loop and the C-terminal regions. The putative 12 transmembrane segments were preserved in HTAU, and the hydropathy plots of these 3 taurine transporters are almost identical. Three potential *N*-glycosylation sites, located in the second extracellular loop, are conserved in the taurine transporters from human, dog and rat. However, an additional potential *N*-glycosylation site in the last extracellular loop was found only in HTAU, suggesting that this site may not be important for the role of taurine uptake. According to the predicted amino acid sequence, the taurine transporter may be a phosphoprotein. Six potential protein kinase C phosphorylation sites and seven potential casein kinase II phosphorylation sites were found in the HTAU protein (Fig. 1). It is noteworthy that protein kinase C activation has been reported to play an important role in the regulation of taurine uptake in cultured renal epithelial cells [13]. Therefore, protein phosphorylation may play an important role in the regulation of the activity of HTAU.

A search of the GenBank showed about 60% amino acid identity, and about 80% amino acid similarity with the dog betaine transporter [14] in the region of residues 27–581. Furthermore, there is 63% identity, and 81% similarity with the rat choline transporter [15] in the residues 193–508. Finally, there is 40–56% identity, and 61–75% similarity with the neurotransmitter transporters [16] in various regions of residues 34–557.

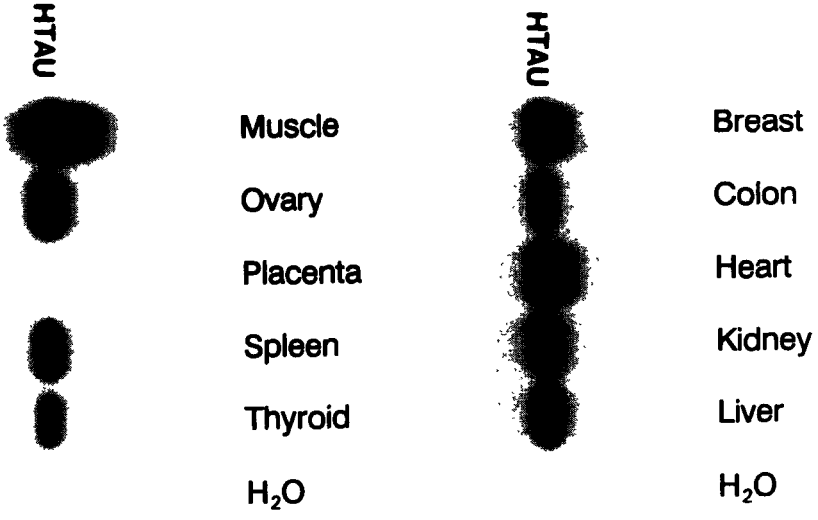

Fig. 2. Tissue distribution of HTAU mRNA as determined by RT-PCR. Three μg of total RNA isolated from each tissue was used for cDNA synthesis in a total of 20 μl reaction volume. However, the RNA isolated from placenta was significantly degraded. One μl of the cDNA reaction mixture was used for PCR amplification (94°C, 30 s, 60°C, 1 min, 72°C, 2 min for 40 cycles), and one third of the PCR product was used for gel electrophoresis. The amplified HTAU products were detected by hybridization with ^{32}P-labelled DNA probes derived from HTAU. An autoradiogram of the Southern blot is shown.

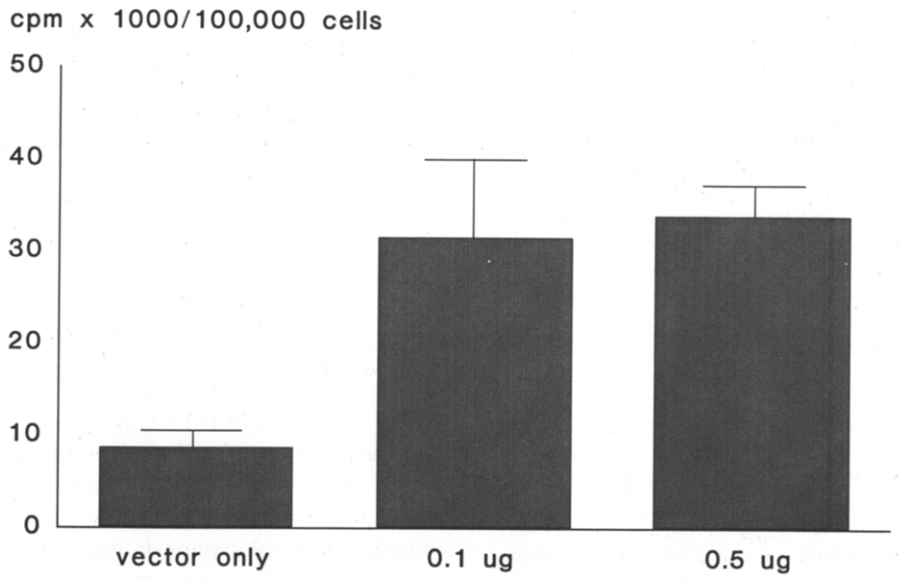

Fig. 3. Transient expression of HTAU in COS-7 cells conferred a higher taurine uptake. Taurine uptake was measured in COS-7 cells, which were transfected with 0.5 μg pSG5 vector, or 0.1 μg, 0.5 μg of the pSG5 carrying the HTAU cDNA. Each bar represents the mean ± standard deviation of triplicates from a single experiment that was repeated with similar results. There is significant difference between vector and HTAU transfected COS-7 cells ($P = 0.006$). However, no significant difference was found between 0.1 μg and 0.5 μg HTAU transfected COS-7 cells.

3.2. *Tissue distribution of HTAU*

As shown in Fig. 2, the HTAU mRNA was detected in all of the human tissues examined, including breast, colon, heart, kidney, liver, muscle, ovary, placenta, spleen, and thyroid. However, the HTAU mRNA represents a rare transcript in all of the tissues examined by Northern analysis, including colon, liver, ovary, spleen and thyroid (data not shown). Recently, it has been shown that protein synthesis and protein kinase C activation, but not RNA transcription, appear to be important for the regulation of taurine uptake in cultured renal epithelial cells [13].

3.3. *Transient expression of HTAU in COS-7 cells confers a higher taurine uptake*

The COS-7 cells transiently transfected with pSG5

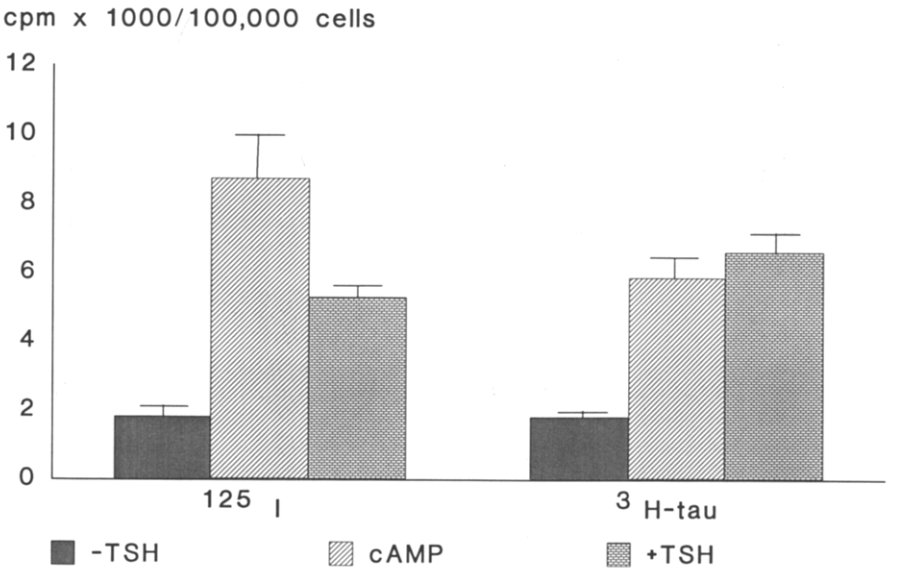

Fig. 4. The taurine uptake in FRTL-5 cells is TSH-dependent, and the TSH-stimulated taurine uptake could be mimicked by exogenous cAMP. For comparison, the iodide uptake was determined in parallel wells. Each bar represents the mean ± standard deviation of triplicates from a single experiment that was repeated with similar results. There is significant difference in iodide uptake among TSH deprived, cAMP treated, and TSH revived FRTL-5 cells ($P < 0.001$). However, in the case of taurine uptake, while the difference between TSH deprived cells and both cAMP and TSH treated cells is significant ($P < 0.001$), the cAMP and TSH treated groups showed no significant difference.

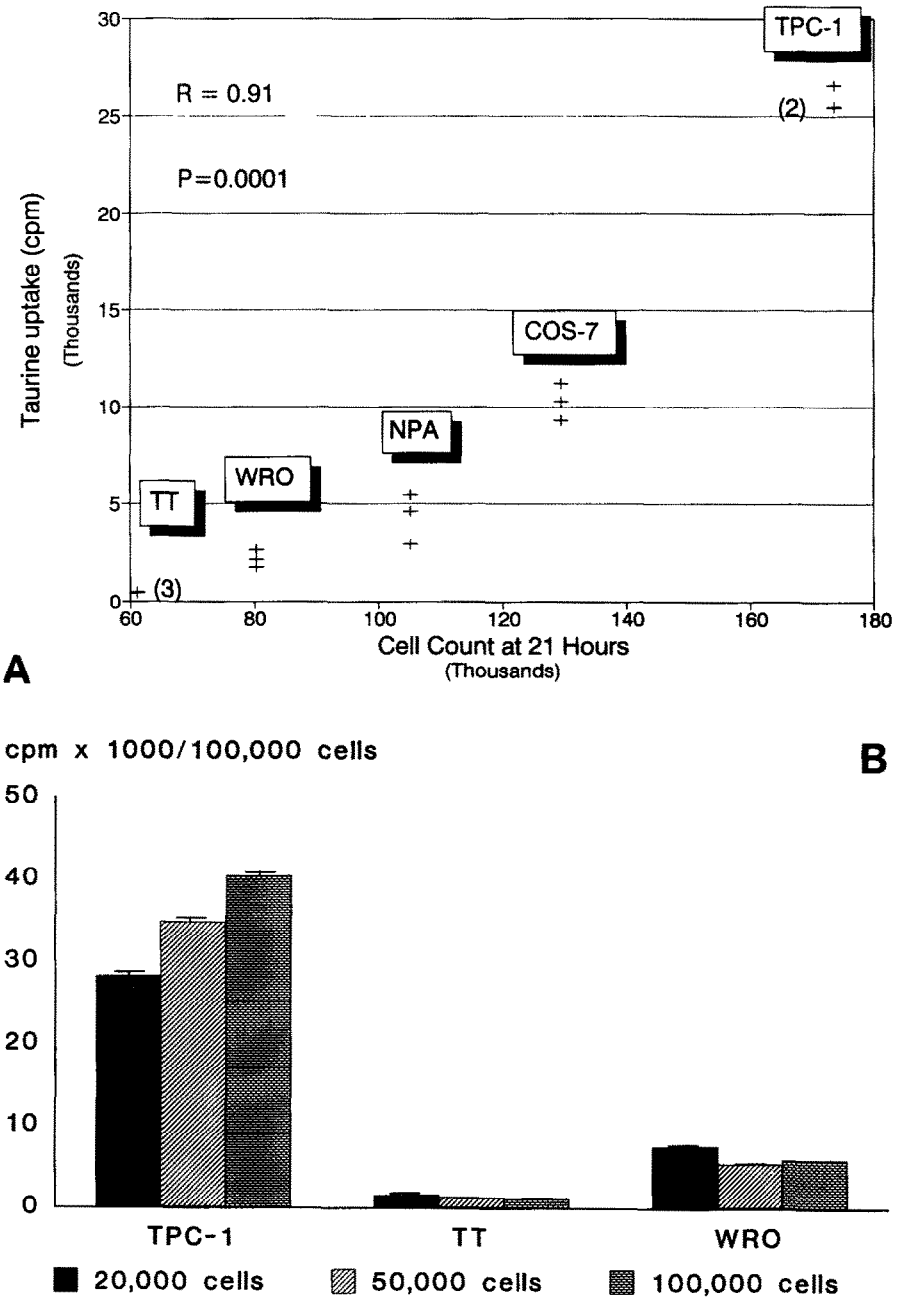

Fig. 5. (A) Correlation between taurine uptake and proliferation rate in TPC-1, COS-7, NPA, WRO and TT cells. *R*, regression coefficient. The uptake assay and cell number counting were performed 21 h after cells were plated at 5×10^4 cells per well. (B) The taurine uptake measured in TPC-1, TT, and WRO cells that were plated in three different cell densities. Each bar represents the mean ± standard deviation of triplicates from a single experiment that was repeated with similar results.

carrying HTAU cDNA accumulated 4–5-fold more [^{3}H]taurine than did control cells (Fig. 3). The control cells include mock transfected, pSG5 vector transfected, and pSG5 carrying antisense HTAU transfected cells. Furthermore, transient expression of HTAU in COS-7 cells did not confer iodide uptake (data not shown), suggesting that the enhanced taurine uptake in the transfected COS-7 cells did not result from non-specific perturbation of the membrane.

3.4. *Taurine uptake in FRTL-5 cells is regulated by TSH through cAMP*

It is known that taurine concentration is high in cells and tissues that possess considerable potential for pro-

ducing oxidants [1]. In the thyroid gland, thyroperoxidase is highly expressed, and is essential for the production of thyroid hormone. Moreover, the administration of thyroid hormone to rats changed the content of taurine in various tissues [17]. However, to date, neither the role of taurine nor its regulation in thyroid gland has been studied. Since TSH is essential for the growth and function of normal thyroid gland, the taurine uptake was investigated in TSH-deprived FRTL-5 cells. Taurine uptake was downregulated by TSH deprivation in FRTL-5 cells. However, the taurine uptake activity in TSH deprived cells was restored almost completely by treatment with 2 mM cAMP (Fig. 4). Similarly, the thyroid-specific iodide uptake has been shown to be TSH-dependent, and the TSH-stimulated iodide uptake could be mimicked by exogenous cAMP in FRTL-5 cells [11]. In the presence of TSH, the addition of cAMP did not increase either taurine or iodide uptake (data not shown). This indicates that both taurine and iodide uptake are regulated by TSH, and largely through cAMP as the second messenger. Further study is underway to determine if HTAU is regulated by TSH in the thyroid gland.

3.5. *Higher taurine uptake may be associated with higher proliferation rate in cultured-cell lines*

The relative taurine uptake activity among several cell-lines was determined. The results showed that the taurine uptake activities in TPC-1, COS-7, and NPA cells are generally higher than in WRO, and TT cells (Fig. 5A). Although the cell density has shown some effect on the taurine uptake activity within the same cells, except TT cells (Fig. 5B, data of COS-7 and NPA cells not shown), the cell density does not appear to have an effect on the relative taurine uptake activity among these cells (Fig. 5B). Interestingly, the proliferation rate of TPC-1, COS-7, and NPA cells is higher than that of WRO and TT cells (Fig. 5A), suggesting that taurine uptake may be associated with proliferation rate. Considering the important roles that taurine plays in membrane stabilization, detoxification, antioxidation, and osmoregulation, higher taurine uptake may be essential to protect the proliferating cells from self-destruction, such as during processes that generate oxidants. In a cell culture system where taurine concentrations can be controlled, taurine has been shown to enhance the proliferation of human lymphoblastoid cells [18] and human retinal pigment epithelial cells [19] in a concentration-dependent manner. It is noteworthy that, for osmoregulation, taurine uptake in kidney cells increases significantly under hypertonic conditions with no change of cell proliferation [7]. Therefore, taurine uptake may be regulated by different factors in different cells.

3.6. *Conclusion*

The primary structure of the human taurine transporter has been determined. Alignment of the human, dog, and rat taurine transporters showed that the taurine transporter is highly conserved among these species. This suggests that taurine plays fundamental roles in mammals. However, to date, it is not clear how taurine serves a variety of functions in different tissues. In dog kidney cells, taurine uptake is regulated by hypertonicity [7]. However, in FRTL-5 cells, taurine uptake appears to be regulated by TSH through cAMP. Although taurine has been shown to stimulate proliferation in some cells [18,19], this study is the first to demonstrate that a higher taurine uptake may be associated with a higher proliferation rate in cultured cell-lines. Finally, cloning of the taurine transporters should open an avenue for the definition of the role of taurine in a variety of tissues.

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