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The taurine transporter gene and its role in renal development

Review Article

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Summary. This paper examines a unique hypothesis regarding an important role for taurine in renal development. Taurine-deficient neonatal kittens show renal developmental abnormalities, one of several lines of support for this speculation. Adaptive regulation of the taurine transporter gene is critical in mammalian species because maintenance of adequate tissue levels of taurine is essential to the normal development of the retina and the central nervous system. Observations of the remarkable phenotypic similarity that exists between children with deletion of bands p25-pter of chromosome 3 and taurine-deficient kits led us to hypothesize that deletion of the renal taurine transporter gene (TauT) might contribute to some features of the 3p- syndrome. Further, the renal taurine transporter gene is down-regulated by the tumor suppressor gene p53, and up-regulated by the Wilms tumor (WT-1) and early growth response-1 (EGR-1) genes. It has been demonstrated using WT-1 gene knockout mice that WT-1 is critical for normal renal development. In contrast, transgenic mice overexpressing the p53 gene have renal development defects, including hypoplasia similar to that observed in the taurine-deficient kitten. This paper reviews evidence that altered expression of the renal taurine transporter may result in reduced intracellular taurine content, which in turn may lead to abnormal cell volume regulation, cell death and, ultimately, defective renal development.

Keywords: Amino acids – (Taurine transporter gene – Regulation – Renal development)

Introduction

Taurine (2-aminoethanesulfonic acid) is a small, sulfur-containing β-amino acid with a sulfonic acid group, rather than the more common carboxylic acid group. In mammals, taurine and inorganic sulfate are the major end products

of sulfur amino acid metabolism. Since taurine cannot be incorporated into protein, it is a prevalent intracellular free amino acid in mammalian tissues. Involved in few biochemical reactions, mainly the conjugation of bile acids to form water-soluble bile salts, taurine nevertheless serves many important cellular functions, including membrane stabilization, antioxidation, detoxification, and cell volume regulation. Arguably, the most important biologic function of taurine is that of osmoregulation. Osmoregulation is critical to both normal brain development and ongoing neural function. In those species where the activity of the rate-limiting enzymes for taurine biosynthesis is either absent (feline) or reduced during infancy (primates – including humans), dietary intake is either essential or conditionally essential, respectively.

Taurine is important in cell volume regulation, maintainance of cell shape, and apoptosis (Law, 1991). Hence, factors that impair the accumulation of taurine into renal cells could potentially result in the death of a significant fraction of undifferentiated cells within the nephrogenic zone of developing kidneys, particularly because of the role of taurine in cell volume maintenance (Law, 1998) and its role in the regulation of cell calcium content (Seabra et al., 1998).

Studies have shown that the renal tubular epithelium controls the total body pool of taurine by adapting to changes in sulfur amino acid availability with changes in the initial rate activity of the NaCl-dependent taurine transporter, specifically located at the brush border membrane surface of the proximal tubule and at the basolateral surface of the distal nephron. However, the role of the taurine transporter gene in kidney development is unclear.

Taurine and kidney development

Taurine has been shown to be essential for the development and survival of mammalian cells, especially cells of the cerebellum and retina (Sturman, 1988). Notably, taurine concentration reaches the millimolar range in the central nervous system (Sturman, 1988). The highest intracellular concentration of taurine is found in neonatal and early postnatal brain, suggesting a key developmental role. For the past decade, the cat has been used as a model to study the role of taurine in development, because activity of the rate-limiting enzyme (cysteine sulfonic acid decarboxylase – CSAD) for taurine biosynthesis is very low in felines and taurine must be supplied from the diet (Sturman et al., 1985). Sturman's group found that the surviving offspring of taurine-deficient female cats have a large number of neurologic defects, including degeneration of retinal pigmented epithelium and ocular tapetum, delayed cerebellar granule cell division and migration, and abnormal cerebral cortical development.

Taurine appears to optimize the proliferation of human fetal cerebral brain cells in culture. Addition of taurine to the medium improves neuron growth, neurite expansion, and neuronal survival (Chen et al., 1998). Human milk contains high concentrations of taurine (Rassin et al., 1977; Rassin et al., 1978). Taurine is considered a conditionally essential amino acid in man, since the enzymatic activity of CSAD, which catalyzes the formation of taurine

from cysteine, is low in humans, and even lower or absent in term and preterm infant liver (Sturman and Gaull, 1975; Sturman et al., 1970; Rassin, 1994). This ontogeny of catalytic enzyme activity is also true in other primates (Sturman et al., 1984). In children with short gut syndrome, taurine is an essential component in total parenteral nutrition (TPN) solutions to prevent a defined retinopathy, which is reversed only by taurine administration. Plasma taurine values in preterm infants fall to almost non-detectable values (Zelikovic et al., 1990), comparable to those in the taurine-deprived cat. Our group has shown that the restoration and maintenance of normal taurine values in preterm infants and infants with short gut syndrome receiving TPN require the specific addition of taurine to TPN solutions (Helms et al., 1995a; Helms et al., 1995b, especially since cysteine is relatively insoluble and, hence, is not a reliable source of taurine.

A recent observation by our laboratory, in collaboration with John Sturman (at the State of New York Basic Science Research Center), in the offspring of inbred, taurine-deficient cats provides evidence for the role of taurine deficiency in renal development and in ongoing kidney damage as well. In these studies, taurine-deficient female cats were bred with taurine-deficient males and, although fetal wastage was high, the surviving kittens showed blindness, ataxia, cerebellar abnormalities and kyphosis, and peculiar facial features, as well as diminished renal size. Histologic examination of the kidneys showed ureteral dilatation, large sclerosed glomeruli, proximal tubular flattening, epithelial atypia, reduced mitochondria at the apex of the tubule, and simplification of the tubule, as compared to age-matched controls. With prolonged taurine deficiency (2 yrs), renal scarring, tubule disorganization and drop-out, and cortical atrophy occur as well as glomerulosclerosis. Figure 1 shows small contracted kidneys from a 2-year-old taurine-deficient cat that show evidence of renal scarring. Diminished renal size in taurine-deficient (0% taurine) versus -sufficient (0.05% taurine) cats is apparent at

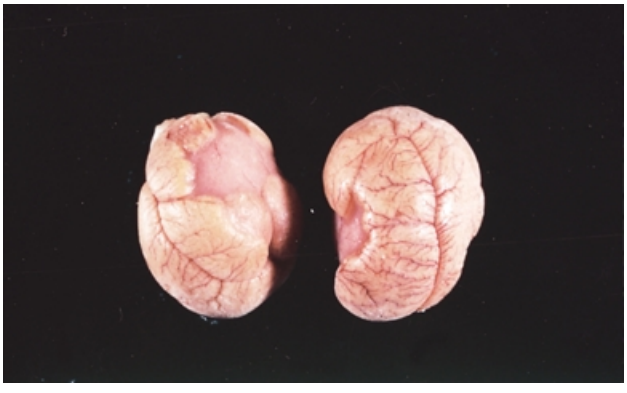

Fig. 1. Small, contracted kidney from taurine-deficient kitten showing renal scarring (left) as compared to normal kidney from age-matched control (right). Reprinted with permission from Kluwer Academic/Plenum Publisher, New York [Han X, Budreau AM, Chesney RW, Sturman JA (2000) Does the taurine transporter gene play a role in 3p-syndrome? In: Della Corte L, Tipton K, Sparagli GP, Huxtable RJ (eds) Taurine 4: Taurine and Excitable Tissues. Kluwer Academic/Plenum Publ, New York (in press)]

8 weeks of age (5.35 ± 0.57 g vs 7.55 ± 0.68 g, $p < 0.05$), and marked in young adult (18 wk) cats (10.86 ± 1.7 g vs 23.05 ± 1.2 g, $p < 0.01$). Distal tubule effects were marked in cats fed the taurine-deficient diet for 2 years. Histologic examination of these kidneys revealed extensive glomerular hypertrophy with interstitial fibrosis. The kidneys of these cats resembled the autopsy findings of a patient with 3p-syndrome (Beneck et al., 1984).

A remarkable similarity exists between human infants with deletion of bands p25-pter of chromosome 3 and taurine-deficient kittens. These children with chromosome 3p-deletions have a specific syndrome of craniofacial manifestations, prenatal growth delay, hypotonia, developmental retardation, cataracts, and cleft palate. In addition, they have renal anomalies including renal hypoplasia, renal malposition (pelvic kidneys) and cortical cysts with increased interstitial connective tissue (Beneck et al., 1984). The facial features of the taurine-deficient kit and the band p25-pter human infant show ptosis, telecanthus, a slight mongoloid slant, down-turned and low-set ears, epicanthal folds and, in some cases, a cleft lip (Mowrey et al., 1993; Narahara et al., 1990; Ramer et al., 1989; Tazelaar et al., 1991; Wieczorek et al., 1997; Witt et al., 1985). Postaxial polydactyly has also been found in both the cat and human conditions. It is important to note that the gene for the taurine transporter has been located on human chromosome 3p21–25 (Patel et al., 1995), suggesting that deletion of the TauT gene might contribute to some phenotypic features of the 3p-syndrome.

Regulation of TauT expression by tumor suppressor gene p53

The p53 tumor suppressor gene product is a sequence-specific DNA-binding protein which acts as a transcriptional activator or repressor of a number of genes (Lin et al., 1994). We have cloned the 5′-flanking promoter region of the rat taurine transporter gene (TauT), and found it contains two perfect p53 DNA binding consensus half-sites, located at −660 to −670 and −896 to −906, respectively. Observations of renal developmental abnormalities in taurine-deficient kittens, and renal hypoplasia and progressive renal insufficiency in p53-overexpressing mice (Godley et al., 1996) led us to propose a role for p53 in TauT expression.

Varmus' group has found that transgenic mice expressing wild-type p53 undergo progressive renal failure through a novel mechanism by which wild-type p53 appears to alter cellular differentiation, rather than by cell cycle arrest or the direct induction of apoptosis (Godley et al., 1996). Our findings suggest that altered expression of certain p53 target gene(s) that are involved in renal development may be responsible for p53-induced progressive renal failure in the p53 transgenic mice.

Regulation of TauT expression by WT-1

Expression of the WT-1 gene is restricted during development to mesenchymal tissues, occurring in specific cells of the collecting system within the

kidney, non-germ cell components of the gonads, uterus, spleen, and mesothelium (Pritchard-Jones et al., 1990). Humans with mutant WT-1 genes demonstrate the Denys-Drash syndrome and the Frasier syndrome, two conditions in which severe renal damage occurs (Pritchard-Jones et al., 1990). Targeted disruption of the WT-1 gene in the mouse results in embryonic lethality in the homozygous state, secondary to the failure of kidney and gonad development (Kreidberg, 1996). However, the precise mechanism by which WT-1 exerts its effects during normal development in the kidney will await identification of actual target genes that are regulated *in vivo*. Therefore, it is of interest to explore the mechanism by which the TauT gene is regulated by WT-1, and the importance of TauT in renal development.

Materials and methods

Regulation of TauT expression by tumor suppressor gene p53

We have directly examined the role of p53 in the regulation of the taurine transporter. A normal rat kidney cell line (NRK-52E) was stably transfected with a temperature-sensitive mutant p53 allele (ts p53 val135), which expresses p53 in a mutant conformation at 37°C and wild-type conformation when cells are cultured at 32°C. We chose to use NRK-52E because expression of p53 was not detectable in this cell line by Western blot analysis employing a polyclonal antibody (Ab-7, Oncogene Research). NRK-52E cells were transfected with msvcl/neo val 135 (a DNA construct containing neo and ts p53 gene) and CMV neo Bam plasmid DNA (vector control) using a lipofectamine transfection kit (Life Technologies, Inc., Grand Island, NY) and selected in 1 mg/ml G418 (geniticin). To standardize the transfection efficiency, 0.1 μg of pRL-CMV vector (pRL Renilla Luciferase control reporter vector, Promega, Madison, WI) was co-transfected in all experiments. Cells were harvested 48 h after transfection and lysed in 200 μl of reporter lysis buffer, and a luciferase assay was performed. G418-resistant colonies (NRK-52E/tsp53) were screened for p53 expression by Western blot analysis. In order to test the role of wild-type p53 in TauT expression, a p53 positive NRK-52E/tsp53 cell line was used in our study. Northern blot analysis was carried out using a specific RNA probe generated from rB16a, rat taurine transporter cDNA (Smith et al., 1992).

Regulation of TauT expression by tumor suppressor gene WT-1

We also examined the effect of WT-1 on TauT expression. An overlapping consensus site (5′-GTGTGGGCGTGG-3′) for WT-1/EGR-1/Sp1 is found in the TauT promoter region (−160 to −171) (Hamilton et al., 1995; Hamilton et al., 1998). To determine if WT-1 and/or EGR-1 play a role in TauT expression, the p-269 promoter reporter, which contains the overlapping site for WT-1/EGR-1/Sp1, was transiently co-transfected with WT-1, EGR-1, or WT1 plus EGR-1 into MDCK cells (Madin-Darby canine kidney, of distal origin), LLC-PK1 cells (porcine kidney, of proximal origin), and NRK-52E cells.

Results

As shown in Fig. 2, switching the incubation temperature of control cells (NRK-52E/neo) from 37°C to 32°C for 24 hours had no effect on expression of TauT. However, expression of TauT decreased about 5-fold (Fig. 2a), and in a time-dependent manner (Fig. 2b), when NRK-52E/tsp53 cells were

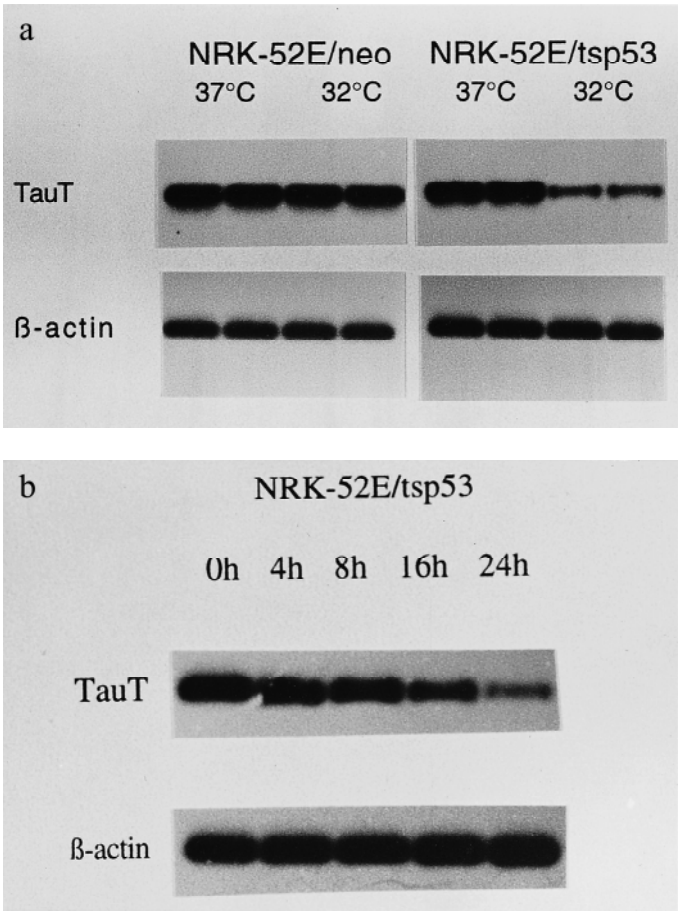

Fig. 2. **a** Northern blot analysis showing regulation of taurine transporter mRNA by p53. Lane 1–2, NRK-52E/neo cells cultured at 37°C; lane 3–4, NRK-52E/neo cells cultured at 32°C; lane 5–6, NRK-52E/tsp53 cells cultured at 37°C; lane 7–8, NRK-52E/tsp53 cells cultured at 32°C. **b** Time course of down-regulation of TauT by p53. β-Actin was used as an internal control for loading

cultured at 32°C, as compared to cells cultured at 37°C. Temperature switching did not affect expression of p53 in NRK-52E/tsp53 cells (data not shown).

Consistent with these findings, taurine uptake was decreased about 5-fold when NRK-52E/tsp53 cells were cultured at 32°C, as compared with cells cultured at 37°C. Temperature switching did not affect taurine uptake by NRK-52E/neo cells which did not contain ts p53 (data not shown). To confirm these observations, the p-963 reporter (containing two p53-binding half-sites and basal promoter sequences) was transiently transfected with or without either wild-type p53 or a mutant p53 (p53–281) into NRK-52E cells. As shown in Fig. 3, wild-type p53 decreased TauT promoter activity approximately 5-fold, whereas mutant p53 had no effect on TauT promoter function. No reduction in promoter activity was evident when p-963 was co-transfected

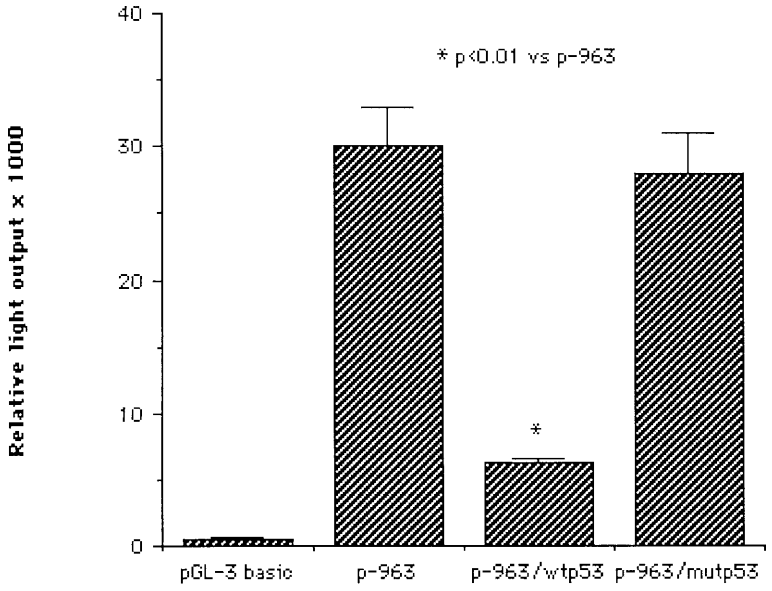

Fig. 3. Regulation of TauT promoter activity by p53. The promoter activity (mean ± SD of 4 samples in relative light units) of each construct is represented by relative light output normalized to pRL-CMV control. The graph represents typical results of four separate experiments

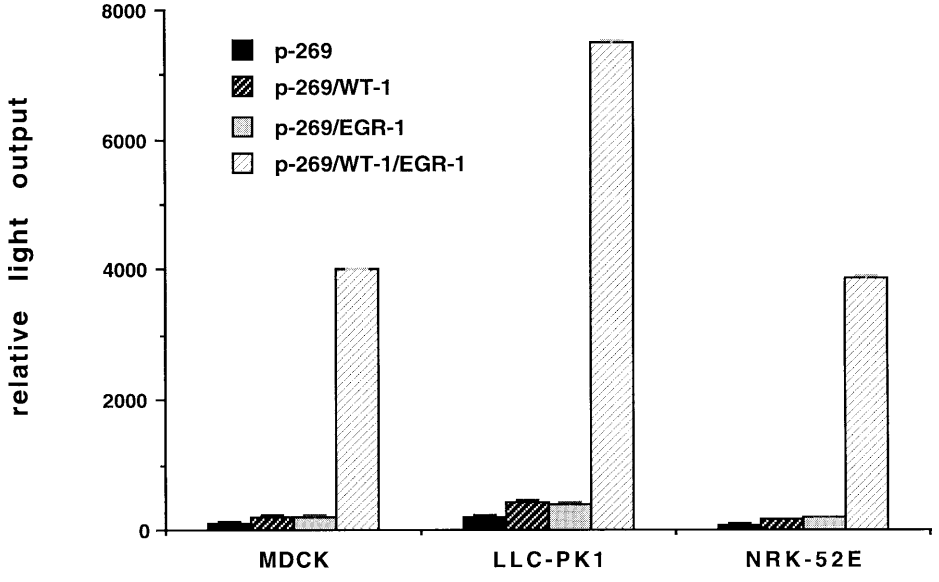

Fig. 4. Regulation of TauT promoter activity by WT-1 and EGR-1. Construct p-269 was co-transfected into MDCK, LLC-PK1, and NRK-52E cells with WT-1, EGR-1, or WT-1 plus EGR-1, and luciferase activity was measured in cell lysates after 24 h. Co-transfection of WT-1, EGR-1, or WT-1 plus EGR-1 had no effect on expression of the pRL-CMV reporter gene (data not shown). The promoter activity (mean ± SD of 4 samples in relative light units) of each construct is represented by relative light output normalized to pRL-CMV control. The graph represents typical results of four separate experiments

with the CMV vector, which does not express p53. Our results demonstrate that the p53 gene down-regulates TauT expression in renal cells.

Figure 4 shows that TauT promoter activity was increased about 2-fold following co-transfection with either WT-1 or EGR-1. However, co-transfection of the p-269 promoter reporter (containing the overlapping site for WT-1/EGR-1/Sp1) with WT-1 plus EGR-1 increased TauT promoter activity by more than 20-fold, suggesting that a synergistic interaction of WT-1 and EGR-1 may play an important role in TauT gene transcription during renal development.

Discussion

In this review we examine evidence that taurine may be an important molecule required for normal renal development. The taurine-deficient cat model displays renal defects that include simplification of the tubular epithelium and renal scarring. Nearly identical renal histopathologic findings are present in the kidneys of the p53-overexpression mouse. We have shown that WT-1 and/or EGR1 are needed for enhanced taurine transporter activity, and others have shown that transgenic mice deficient in WT-1 have renal abnormalities similar to p53-overexpression mice. Finally, the rare human condition with deletion of 3p-25pter, the portion of the 3rd chromosome containing the taurine transporter, is noteworthy for renal maldevelopment. We speculate that these models have in common abnormal renal taurine content during *in utero* development, and, accordingly, that taurine and its normal uptake into developing renal cells is required for normal renal development.

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