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Purpose. Using cultured human lens epithelial cells (HLECs) and bovine lens epithelial cells (BLECs) and the nature of the relationship between taurine-concentrating capability and intracellular polyol accumulation or extracellular hypertonicity.

Methods. The kinetic characteristics of taurine accumulation based upon the measurement of *in vitro* [^{3}H]-taurine uptake were compared in cultured HLECs and BLECs pre-exposed to either galactose-supplemented medium or extracellular hypertonicity

Results. The capacity to accumulate [^{3}H]-taurine was significantly lowered after chronic (20 hour) incubation of cultured BLECs in 40 mmol/l galactose in contrast to HLECs. Inhibition of the *intracellular* taurine transport site appeared to be noncompetitive as there was a marked reduction in the V_{max} without significant alteration in the Km. Galactitol content in BLECs exceeded five times that found in HLECs. The coadministration of the aldose reductase inhibitor, sorbinil, with 40 mmol/l galactose completely prevented the inhibitory effect of galactose on [^{3}H]-taurine uptake. Acute exposure (3 hours) of HLECs and BLECs to a range of 10 to 40 mmol/l galactitol or 10 to 40 mmol/l galactose plus sorbinil-supplemented medium suggested by Dixon plot analysis that neither galactitol nor galactose interacted with the *extracellular* taurine transport site.

In contrast, [^{3}H]-taurine accumulation was markedly elevated in both HLECs and BLECs after chronic exposure to galactose-free medium made hyperosmotic by supplementation with sodium chloride. The enhanced taurine uptake capacity involved an increase in V_{max} without significant change in the Km value.

Conclusions. These results demonstrate lens epithelial cells express a taurine transporter protein capable of active uptake but predisposed to inhibition by *intracellular* galactitol when the sugar alcohol is present in high enough concentration to interfere with cell metabolism. Furthermore, lens epithelial cells respond to hypertonic stress by raising taurine transport activity.

OSMOREGULATORY ALTERATIONS IN TAURINE UPTAKE BY CULTURED HUMAN AND BOVINE LENS EPITHELIAL CELLS

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OSMOREGULATORY ALTERATIONS IN TAURINE UPTAKE BY CULTURED HUMAN AND BOVINE LENS EPITHELIAL CELLS

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CHAPTER I

Introduction

The eye begins its development at about two weeks after conception. A detectable thickening of neural ectoderm[1] eventually leads to the maturation of our most recognizable of sense organs. The eye could quite possibly be the major sense organ from which we absorb and process environmental stimuli. From the day we are born, vision allows us to respond to our environment and alter it to meet our own needs, either out of necessity or for artistic impressions. Is it then any wonder why any affliction or detrimental action toward the eye would cause great distress? The ability to maintain our sight is the focus of research dealing with such complications as cataracts and glaucoma.

Cataracts and Lens Anatomy

One common and devastating affliction to the eye is the cataract. A cataract is formed when the lens of the eye loses its consistency, begins to harden, and becomes cloudy. This cloudiness has been compared to a smudged pair of glasses. This cloudiness causes a dim and distorted image to be formed on the retina, thus decreasing vision clarity. The most common way of treating cataracts is through surgical removal of the clouded lens and replacing it with an artificial lens.

In order to discuss the nuances of cataract formation it is necessary to have some knowledge of the anatomy of the lens. The lens itself originally develops from a thickening of neural ectoderm cells that internalize and then eventually "pinch-off" to form a *lens vesicle*. Shortly after the formation of this lens vesicle, the posterior cells from within this structure begin their elongation. These elongated cells are now referred to as primary fiber cells and progressively begin to lose their nuclei. The anterior portions of the lens remains unchanged, other than the fact that they become increasingly cuboidal, and are then referred to as the lens epithelium. All future growth of the lens is reliant upon this portion of the lens.

The lens epithelium is located only on the anterior portion of the lens. It is structurally a monolayer of squamous cells, and are held together primarily by desmosomes.[2] These cells also contain a large amount of interdigitations.[3] The underlying lens fiber cells are very tightly packed, but are able to maintain optical transparency due to their geometrical arrangement.[1] Some cataracts are formed by the disruption or this arrangement due to trauma, or physical laceration, to the lens itself.[4] Others, however, are formed by a coagulation of molecules within the anucleate fibrous lens cells. These lens cells are constantly being replaced by the epithelial lens cells, which are constantly undergoing mitosis. Because these fiber cells lack any nuclei, most regulation (protein synthesis) is taking place within the epithelial lens cells. So it is there that we will concentrate our study.

The true cause of a cataract is unknown. The possibilities are many. They can be formed due to injury (traumatic cataract), infection, or even blood disorders. Cataract formation could also be due to the aging process itself. The most common cause of

cataract formation, however, is diabetes. There are three basic types of cataracts: 1) Nuclear – opacity is formed in the center of the lens, 2) Cortical – opacity forms at the cortex of the lens with spokes that extend from the outside to the center of the lens, and 3) Subcapsular Cataract – the opacity appears in the back of the lens.[4] It is the cortical and subcapsular cataracts that are most prevalent in those individuals suffering from diabetes.

Diabetic Cataract Formation and the Sorbitol Pathway

Individuals with diabetes are at a high risk for the development of cataracts. Studies have shown, however, that control of the disease can greatly reduce cataract formation, but in no way eliminates it. Studies of cataracts associated with diabetes have shown disruption of several metabolic pathways associated with high glucose metabolism possibly due to a high intracellular glucose level. Diabetic cataracts are generally cortical as well as subcapsular opacities. Both juvenile (type 1) and adult onset diabetes (type 2, diabetes mellitus) forms these types of cataracts. These opacities usually appear in both eyes at about the same time. The rate of development of diabetic cataracts is largely dependent upon the severity and prolonged poor control of the disease.[4]

Diabetes associated cataractogenesis is possibly initiated by osmotic stress caused by intralenticular accumulation of polyols produced by the enzyme aldose reductase.[5] There are three basic types of cataractogenic sugars which are as follows: xylose, galactose, and glucose. These sugars enter the lens and are eventually converted into their respective sugar alcohol in the presence of aldose reductase (figure 1). Glucose is

converted into the sugar alcohol sorbitol. The stress the cell receives from the accumulation of sorbitol is eventually relieved as it is eventually converted to fructose by means of sorbitol dehydrogenase. However, galactose is converted into galactitol and undergoes no further conversion, and therefore this polyol remains in the lens unable to be metabolized any further. This is supported due to the fact that galactose induced cataract formation occurs much more rapidly than those fostered by high glucose content.[8]

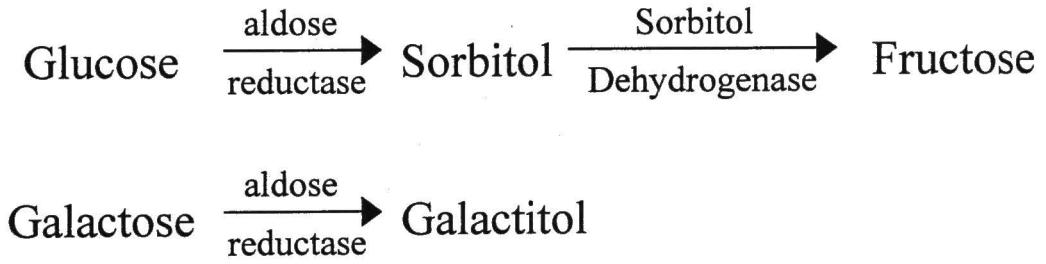

Figure 1. Conversion of sugars into their respective sugar alcohol

These polyols accumulate due to the fact that the lens membrane is very poorly permeable to these sugar alcohols.[6] While the sorbitol pathway is constantly active, it is the high intracellular glucose concentration, as in the case of diabetes, that increases the production of the polyols. The pathway then, is always saturated and the time it take the sortbitol to be removed via sorbitol dehydrogenase is ever increasing.

The lens itself maintains a high intracellular potassium concentration [K+] and a low sodium concentration [Na+]. This gradient is kept stable by a Na^+-K^+ pump, which actually pumps potassium in and sodium out of the cell. The lens must maintain a source of ATP in order to create this gradient.[7] It is this gradient that must be sustained in order

to preserve osmotic balance. Molecules that interrupt this process that maintains the unbalanced ion concentration can cause osmotic swelling, which is the possible cause of cataractogenesis.

An accumulation of polyols is then followed by an influx of water causing the osmotic swelling due to the increased concentration of the sugar alcohols, or hypertonicity, within the lens. It is this increased swelling and eventual rupture of the lens fibers that is believed to be responsible for the diabetic cataract formation.[8]

Taurine as an Osmolyte

Osmolytes are present in cases where a cell has experienced an extreme extracellular perturbance. The cells are able to maintain constant volume due to influx, or efflux, of these inorganic and organic solutes.[9] Such compounds are accumulated during hypertonic cell shrinkage and released during hypotonic cell swelling as is shown in Figure 2. These are integral transitory phases in the process of regulatory volume increase and decrease respectively.[9]

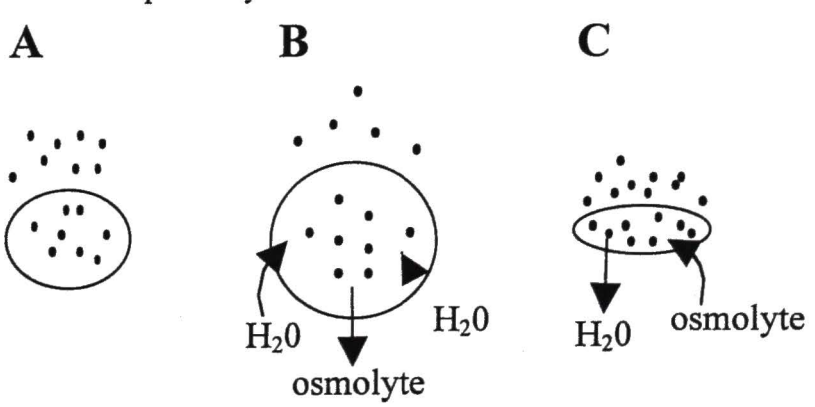

Figure 2. Illustrations showing cells in (A) isotonic, (B) hypotonic, and (C) hypertonic extracellular conditions

Certain amino acids, such as taurine, as well as some polyols and methylamines have been characterized as organic osmolytes. These substances most likely found evolutionary significance in the high salt environment of the oceans and seas.[10] The cells do not accumulate inorganic ions such as K+ and Na+ due to the simple fact that salts interfere with many enzymatic rates.[7] This is shown in a change in the Michaelis constant Km. The metabolic function and transmembrane potentials would be severely retarded.

Taurine is still a major osmolyte in many marine animals, but it has also been identified in many of our own tissue systems. This is especially true for the kidney[11] and liver.[12] Many studies have been performed on the kidney in respect to osmotic stress and taurine uptake. It have been shown that the taurine transporter adaptively regulates itself to the amount of taurine in the external environment. As the external concentration increases, there is a subsequent down regulation in the transporter mRNA and subsequently, its corresponding protein.[13]

Structure and Function of Taurine

The effect of taurine has been examined in a variety of biological systems. In order to gain a greater understanding of the implications of taurine's presence in the lens epithelial cell, it is necessary to examine the biological effects it elicits elsewhere. The role of other osmolytes, such as myo-inositol, has been researched in the bovine lens epithileal cell.[14] However, the role of taurine, as well as the kinetics of its transport, have not been elucidated in any prior research study.

Taurine is regularly consumed in a normal diet, however it can also be generated from the metabolism of cyteine. Much of the research dedicated to taurine has been directed toward this pathway. The rate limiting and highly regulated enzyme cysteine dioxygenase is recognized as the beginning of this metabolic pathway (figure 3).[15] Pyruvate as well as acetaldehyde are also derivatives of this pathway and are not to be incorporated into the final taurine structure.[16]

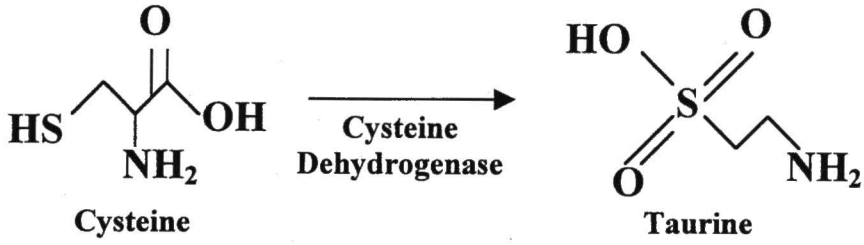

Figure 3. Metabolization of cysteine into taurine

Taurine has been shown to have a role in the possible reversal of arrhythmias associated with epinephrine in the heart[17] and in maintaining calcium homeostasis.[18] It is the most abundant free animo acid in cardiac tissue and has been suggested to promote recovery in congestive heart failure.[19] The effect and abundance of taurine on the cardiac system is evidence enough to show biological significance and promote its further study.

The high concentration of taurine in the mammalian central nervous system has spawned research hypothesizing its possible actions as a neurotransmitter or regulator.[20] More evidence has been accumulated relating taurine's involvement in mammalian retinal development in rhesus monkeys.[10] Taurine exclusion from a pregnant feline female results in newly born kittens that have severe neurological deformities or females

that fail to produce any offspring at all.[21] The importance of taurine in early development is shown through both of these models.

In other tissue systems, taurine appears to be adaptively regulated. The extracellular concentration of taurine is able to influence the uptake of taurine into the cell, meaning that uptake is concentration dependent. It has been shown that a high extra-cellular taurine concentration significantly decreases uptake as well as decrease the transcription of mRNA for the taurine transporter.[22]

The taurine transporter itself has been shown to be sodium dependent in the kidney cells, increasing uptake under chronic hypertonic conditions.[13] Studies have shown that cellular depletion of ATP completely inhibits taurine transport.[23] Thus, it appears that ATP mediates the passage of taurine into the cell making this an active transport system, dependent on both energy and sodium.

The Taurine Transporter

The taurine transporter itself has been studied in several systems such as renal development, human thyroid function, and rat brain. The taurine transporter has been characterized as a saturable, high affinity, Na^+ - and Cl^- -dependent β-system and accounts for the taurine accumulation in most systems.[24] The β-system basically includes a family of very similar Na^+ coupled transport systems, each with 12 hydrophobic transmembrane segments.[25] There is also a swelling-activated taurine channel that accounts for the net loss of cellular taurine following hypertonic cell swelling.[26]

It has been demonstrated that binding of Na+ to this β-system was a prerequisite for for the binding of taurine to this transporter. It has also been shown that there was no influx in the absence of extracellular Na+.[24] In fact, it was found that intracellular [Na+] actually increased taurine efflux from the cell, while an increase in extracellular [Na+] stimulated taurine influx.

Reports have also shown that the taurine transporter has numerous phosphorylation sites. Protein kinase C (PKC) leads to the phosphorylization and inactivation of the β-system in a human choriocarcinoma cell line.[27] This is important, as PKC can be activated by cell swelling, or axial stretching, thus blocking the influx of taurine. An increase in cAMP however, is reported to stimulate taurine influx in rat heart cells.[28]

Transport Kinetics

It is now necessary that we analyze the movement of these molecules into and out of the lens cell and the subsequent quantification of that movement. Substrate transport and action can normally be analyzed by methods similar to that of enzyme kinetics. The taurine transporter is not an exception. The changes in the apparent affinity and rate of flux of taurine can be accurately measured as well as stimulation or inhibition of flux rate and affinity. Transporter systems commonly show saturation kinetics, and can be graphed as a curve such as that shown in figure 4.

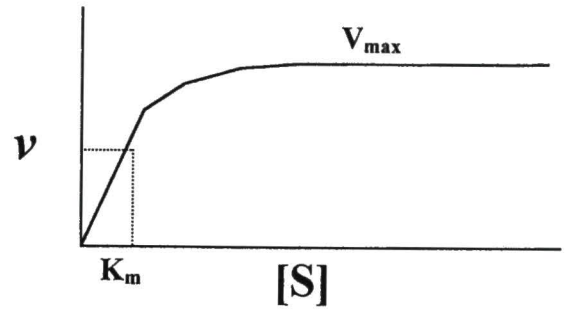

Figure 4. Saturation velocity curve with the substrate labeled [S] on the x-axis and velocity, v, on the y-axis

Transporters that exhibit this kind of characteristic can be analyzed by the Michaelis-Menton equation. The application of this equation can give us such useful parameters as Km and Vmax. The equation is as follows:

$$v = \frac{V_{max}\ [S]}{K_m + [S]}$$

The value of Vmax is defined as the maximum velocity, or the highest activity (rate of flux) of the transporter. This is the concentration point at which the transporter is almost completely saturated with substrate [S]. Any increase in the substrate concentration does little or nothing to increase the velocity 'v' thereafter. At this point a maximal theoretical value for velocity is attained. This point is further described as being zero order kinetics where 'v' is essentially equal to Vmax.

The Michealis-Menton constant, Km, is the substrate concentration at which the velocity is equal to one-half the Vmax. It is when the substrate concentration is close or equal to the Km value that the velocity is extremely responsive to slight variations in the substrate concentration. Km can be seen as the "apparent" affinity for a given substrate.

The value of Km is commonly close to the physiological concentration of the substrate. When substrate concentration is much less than the Km, changes in velocity are much more dependent on this concentration. It is in this area of the velocity curve that is referred to as "first order kinetics" and its equation can be expressed by removing the substrate [S] from the denominator. The modified equation is written as follows:

$$v = \left(\frac{V_{max}}{K_m} \right) [S]$$

The rate at which a molecule is acted upon by a transporter, can be influenced by an inhibitor. Two common types of inhibition are competitive and noncompetitive. The effect of competitive inhibitors, as seen in figure 5, is an

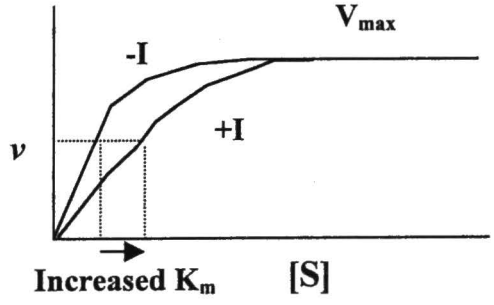

Figure 5. Competitive inhibition showing the increase in K_m with no change in V_{max}. The control is denoted (-I), while inhibition is represented with (+I).

increase in K_m. This graph shows an apparent decrease in substrate affinity in the presence of inhibitor, meaning it now takes higher substrate concentration to reach the same velocity. The inhibitor molecule actually binds to the site on the transporter that is the binding site for the substrate. The highest rates of inhibition will occur when either there is a high inhibitor concentration, or a low substrate concentration with respect to the inhibitor. This is represented graphically in

figure 5 where –I denotes control and +I denotes the presence of a single fixed concentration of inhibitor. Conversely, noncompetitive inhibition retains the same Km, while there is a decrease in the $V_{max,}$ which is shown in Figure 6. As the concentration of inhibitor is increased, the V_{max} is decreased, i.e. the maximal rate of flux or transport is decreased with no change in the affinity for the substrate.

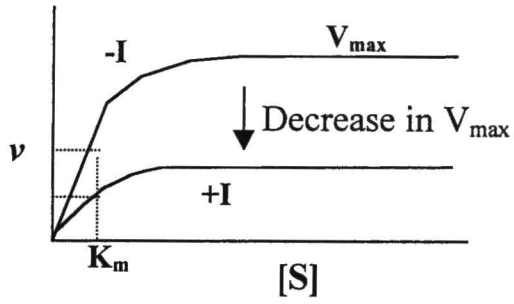

Figure 6. Noncompetitive Inhibition showing the decrease in Vmax with no sucequent change in Km. The control is denoted (-I), while inhibition is represented with (+I).

In this study we will also use Dixon plots in order to show possible inhibition. The reciprocal of the uptake velocity is plotted against increased concentrations of the possible inhibitor. From this data, it can then be determined if there is possible inhibition and what type, either competitive or noncompetitive inhibition. Examples of each are seen in figure 7.

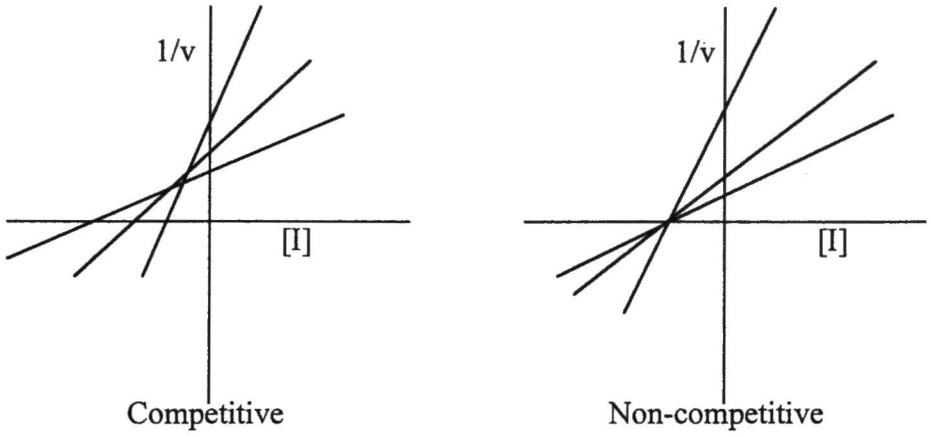

Figure 8. Visualization of competitive and non-competitive inhibition as shown by Dixon Analysis. Lines intersecting above the x-axis in the upper left quadrant indicate competitive inhibition. Lines intersecting directly on the x-axis are indicative of non-competitive inhibition.

SPECIFIC AIMS

1. Determine the kinetic parameters of taurine transport using both cultured human lens epithelial cells (HLE-B3) and bovine lens epithelial cells (BLEC) under hypertonic and physiologic conditions.
2. Determine the kinetic parameters of taurine transport for both cultured human lens epithelial cells (HLE-B3) and bovine lens epithelial cells (BLEC) after chronic exposure to galactose or galactose plus the aldose reductase inhibitor, sorbinil, in order to examine the effects of the sugar alcohol, or sugar, on [^{3}H]-taurine uptake.
3. The effects of increased concentrations of galactose or galactitol on [^{3}H]-taurine uptake in both cultured human lens epithelial cells (HLE-B3) and bovine lens epithelial cells (BLEC) will be plotted by Dixon analysis and examined for either competitive or noncompetitive inhibition.

CHAPTER II

The research in this section has been included in the manuscript

Osmoregulatory Alteration in Taurine Uptake by Cultured Human and Bovine Lens Epithelial Cells

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INTRODUCTION TO THE STUDY

The lens can counterbalance water stress either by accumulating osmotically active, nonperturbing organic osmolytes (regulatory volume increase) or by release, from cell to medium, of organic osmolytes (regulatory volume decrease).[19,29] To date, three compatible organic osmolytes have been identified in cultured lens epithelial cells, sorbitol, myo-inositol and taurine, the latter being an amino acid derivative and the focus of this study.

As a putative lens osmolyte, taurine could participate in volume regulation when the lens epithelial cell experiences osmotic perturbation. Active uptake of taurine into the cell (influx) occurs through a Na^+ and Cl^- dependent taurine transporter.[25,30] The mechanism of passive movement of taurine out of the cell (efflux) is controversial. To date it remains an open question as to whether the taurine efflux pathway occurs through a volume-

sensitive organic osmolyte anionic (chloride) channel or if volume-activated taurine efflux is via a pathway independent of volume-sensitive anion channels.[31-34]

In order to identify the cellular mechanisms, by which experimental diabetes, coincident with polyol accumulation and taurine depletion impairs normal lens function, a reliable set of *in vitro* cellular parameters should be identified. I previously reported that galactose inhibits the ouabain-sensitive uptake of myo-inositol.[35] The coadministration of the aldose reductase inhibitor, sorbinil, to galactose-supplemented medium corrected the attenuated myo-inositol uptake, indicating that galactitol interferes with the myo-inositol transport system.

The studies reported herein were designed to investigate the effects of high extracellular galactose on [^{3}H]-taurine uptake using a range of taurine concentration from 1.5-400 μmol/l, as well as the influence on taurine uptake by exposure to hypertonic medium. Experiments such as these, employing a concentration range of taurine and hyperosmotic insult, are necessary to uncover potential relationships between elevated intracellular polyol content, hyperosmolarity and taurine accumulation.

MATERIAL AND METHODS

Cell Culture

Bovine eyes obtained from a local slaughterhouse were brought on ice to the laboratory where the lenses were removed aseptically. Bovine lens epithelial cells (BLECs) were isolated and cultured in 10% bovine calf serum supplemented Eagles minimal essential medium (MEM, 257±2 mosm) as previously described.[36] Human lens epithelial cells

(HLE-B3 and hereafter referred to as HLECs) were obtained from Dr. Usha Andley and cultured in 20% fetal bovine serum supplemented Eagles minimal essential medium. All studies with BLECs were performed on second passage cells, whereas experiments with the immortalized HLECs were started at passage 11 and did not exceed passage 16.

Taurine Accumulation

The effect of chronically exposing (hereafter operationally defined as a 20 hour incubation period in serum-supplemented medium) cells to 40 mmol/l D-galactose on [^{3}H]-taurine accumulation was carried out as follows. The cultured cells were divided into groups in 25 cm^2 flasks and the medium replaced with physiological medium containing 5.5 mmol/l glucose or 40 mmol/l galactose (Sigma Chemical, St. Louis, MO) in the presence and absence of 0.1 mmol/l sorbinil (Pfizer, Groton, CT) for 20 hours. Thereafter, the cells were rapidly rinsed by, and then switched into, a simpler uptake medium (medium A). Medium A consisted of the following, 5.5 mmol/glucose, 135 mmol/l NaCl, 5.4 mmol/l KCl, 1.8 mmol/l $CaCl_2$ and 10 mmol/l N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid (HEPES), pH 7.4. Studies with BLECs used a medium A that was serum-free. For studies with HLECs, medium A contained 0.5% fetal bovine serum (because the cells would not remain anchored to the tissue culture flasks for the three hour taurine uptake period in serum-free medium). Cells maintained in physiological medium or 40 mmol/l galactose were rapidly rinsed in galactose-free, medium A (containing 5.5 mmol/l glucose); cells previously maintained in 40 mmol/l galactose plus 0.1 mmol/l sorbinil were rinsed in galactose-free, medium A (containing 5.5 mmol/l glucose) plus 0.1 mmol/l sorbinil. Cell cultures were then switched to fresh

medium A containing trace amount of [^{3}H]-taurine (1.0 μCi/ml, Amersham, Arlington Heights, IL) over a concentration range of 1.5-400 μmol/l taurine for a three hour uptake period at 37°C. After isotope incubation, the medium was removed and the cell monolayers prepared for liquid scintillation counting as previously described.[14] Replicate 1.0 ml aliquots were taken for liquid scintillation counting (Packard). Triplicate 25 μl aliquots were taken for protein determination by the method of Bradford [37] with bovine serum albumin (Sigma Chemical, St. Louis, MO) as standard.

Experiments for Dixon plot analysis were performed as follows; cells previously maintained in physiological medium were rapidly rinsed with medium A before being divided into four groups: glucose-free medium A containing 10, 20, 30, and 40 mmol/l galactose. The experiment was performed at a fixed osmolarity of medium A as a fructose supplementation was reduced accordingly as the galactose concentration was raised from 10 mmol/l to 40 mmol/l. All experimental sets contained 0.1 mmol/l sorbinil. Taurine accumulation was followed by the addition of [^{3}H]-taurine (1.0 μCi/ml) at concentrations of 1.57, 6.25, and 25 umol/l taurine for a 3 hour uptake period at 37°C. Essentially the same experiment was performed with 10, 20, 30 and 40 mmol/l galactitol with the exception that, in this situation, the medium A also contained 5.5 mmol/l glucose. This experiment was also performed at a fixed osmolarity by fructose supplementation and sorbinil was omitted during the uptake period.

The effect of chronic exposure of cells to hypertonic medium on [^{3}H]-taurine uptake was conducted as follows. Cells were maintained in physiological medium (257 ± 2 mosm) or physiological medium made hyperosmotic by the addition of 116 mmol/l NaCl (hypertonic medium A, 473 ± 6 mosm) for 20 hours. The cells were then switched to

medium A or hypertonic medium A with a trace amount of [^{3}H]-taurine (1.0 μCi/ml) over a concentration range of 1.5 to 400 μmol/l taurine for a three hour uptake period at 37°C. The osmolarity of the hypertonic-adjusted medium was determined with a vapor pressure osmometer (model 5500; Wescor, Salt Lake City, UT). A three-hour [^{3}H]-taurine uptake period was chosen for the velocity studies subsequent to determination of a linear time course of taurine uptake. For the determination of linear taurine uptake, the experiment was carried out essentially as described above with the exception that medium A or hypertonic medium A contained a fixed concentration of 100μmol/l taurine and triplicate flasks were collected at 1, 2, 3, 4, 6, and 8 hours.

Determination of intracellular galactitol

The concentration of intracellular galactitol was resolved by anion exchange chromatography and pulsed chase electrochemical detection using a Dionex BioLC chromatography system (Dionex, Sunnyvale, CA) as previously described.[14] Human and bovine lens epithelial cells were grown to confluence in 150-cm^2 flasks in physiological medium and switched to physiological medium or 40 mmol/l galactose-containing medium for 20 hours before dispersion with trypsin in serum-free physiological medium (MEM) and centrifuged at 2,500g at 4^{0}C for 8 min. The cells were suspended in 0.9 ml of 0.3 N zinc sulphate (Sigma Chemical, St. Louis, MO) and rapid freezing in liquid nitrogen and thawing at 37^{0}C for three repetitions accomplished cell disruption. The samples were transferred to a 5-ml Dounce homogenizer and subjected to five strokes. The homogenate was centrifuged at 18,000g at 4^{0}C for 20 min, and the cell pellet was saved for protein determination. The supernatant was adjusted to 1 ml with 0.3 N zinc sulphate and precipitated with 1.0 ml of 0.3 N barium hydroxide sulphate (Sigma

Chemical, St. Louis, MO). The precipitate was centrifuged at 2,500g at 4^0C for 8 min and the supernatant was removed and stored without further modification at -20^0C for subsequent galactitol analysis.

Calculations and Statistical Analysis

Calculations to determine Km and Vmax were performed using TableCurve (Jandel Scientific) Software. Appropriate statistical analyses were applied to each group of data as indicated.

RESULTS

Chronic Effect of 40 mM Galactose and Aldose Reductase Inhibition on Taurine Uptake

Cultured human and bovine lens epithelial cells responded with a rapid accumulation of taurine for up to eight hours in a fixed concentration of 100 µmol/l taurine containing a trace amount of [^{3}H]-taurine (Fig. 1). Once an appropriate time for uptake studies was determined from a linear region of figure 1, the chronic effect of extracellular galactose on taurine uptake was investigated. Cultured HLECs were exposed to 40 mmol/l galactose in the presence and absence of 0.1 mmol/l sorbinil for 20 hours. After being switched to *galactose-free* medium A with and without sorbinil, [^{3}H]-taurine uptake was followed for a 3-hour period over a concentration range of 1.5 to 400 µmol/l taurine. Correspondent control cells were maintained in physiological medium and [^{3}H]-taurine uptake was likewise followed. The accumulation curve for [^{3}H]-taurine for cultured HLECs was indistinguishable irrespective of whether the cells were preincubated in physiological medium, 40 mmol/l galactose or 40 mmol/l galactose plus sorbinil (Fig.

2A). On the contrary, the uptake of [^{3}H]-taurine was reduced after chronic exposure of cultured BLECs in 40 mmol/l galactose (Fig. 2B). The coadministration of sorbinil with 40 mmol/l galactose completely prevented the inhibitory effect of galactose on [^{3}H]-taurine uptake. Neither human nor bovine lens epithelial cells showed a statistically significant change in Km for taurine transport under any experimental condition (Table 1) but a significant change of V_{max} was observed for the BLECs exposed to extracellular galactose.

Intracellular galactitol levels were determined by anion exchange chromatography for human and bovine lens epithelial cells maintained in physiologic medium (Phys, 257 ± 2 mosm) or high extracellular galactose medium, Gal (Phys + 40 mmol/l galactose, 285 ± 4 mosm) for 24 hours. As shown in Table 2, intracellular galactitol was dramatically increased in BLECs maintained in Gal, the polyol level being greater than 325 nmol/mg protein after a 24 hour incubation period. By contrast, intracellular galactitol content in HLECs was five times lower, not exceeding 60 nmol/mg protein, under identical culture conditions. The difference in accumulated polyol content likely reflects the relative levels of aldose reductase activity between the two species of cultured cells.

Acute Effect of Galactose and Aldose Reductase Inhibition on Taurine Transport Activity

Human and bovine lens epithelial cells were briefly exposed to 10 to 40 mmol/l galactose containing the inhibitor of galactitol biosynthesis, sorbinil. By applying this experimental approach, the acute (3-hour exposure) effect of galactose on taurine transport could be analyzed without interference from accumulated intracellular

galactitol. Figure 3A is a Dixon plot resulting from the acute exposure of HLECs with D-galactose and sorbinil using three random doses of taurine at 1.57 umol/l, 6.25 umol/l, and 25 umol/l and a trace amount of [^{3}H]- taurine. The failure of the Dixon plot to intersect for all values of substrate verifies that D-galactose does not interact with the taurine transport system in human lens epithelial cells. The data shown in figure 3B was likewise generated using BLECs. These data, as with the HLECs, demonstrated that D-galactose had no inhibitory effect on taurine uptake in bovine lens epithelial cells.

Acute Effect of Galactitol on Taurine Transport Activity

The effect of high extracellular galactitol on taurine transport was also examined by Dixon plot analysis. Figure 4A is a Dixon plot of [^{3}H]-taurine uptake resulting from an acute (3-hour) incubation of HLECs to galactitol. The concentrations of galactitol were 10, 20, 30 and 40 mmol/l and the taurine concentrations were as above, 1.57 umol/l, 6.25 umol/l, and 25 umol/l with a trace amount of [^{3}H]- taurine included in the uptake media. The Dixon plot analysis of the data showed no intersection of lines for the human lens epithelial cells (Fig. 4A). Therefore, as with galactose, exogenous galactitol has no acute inhibitory effect on taurine transport. The same conclusion was achieved using bovine lens epithelial cells (Fig 4B) in place of human lens epithelial cells.

Hypertonicity-Induced Enhancement of Taurine Uptake

Human and bovine lens epithelial cells were pre-exposed to a NaCl-supplemented hyperosmotic environment for 20 hours. The addition of sodium chloride promoted enhanced taurine accumulation in cultured human and bovine lens epithelial irrespective

of whether a fixed concentration of taurine was examined (Fig. 1) or if a 3-hour uptake period over a dose range of 1.5 to 400 μmol/l taurine was considered (Fig. 5). The kinetic character of [^{3}H]-taurine uptake was to increase the V_{max} without significant alteration to the Km (Table 3).

DISCUSSION

The nature of the taurine transport system has been reported in monolayer cultures of several human cell lines, including; colon carcinoma[38], retinal pigment epithelium[39] and glioma.[40] The transport process is typified by a single saturable high affinity site with a Michaelis-Menten constant ranging from 2 to 11 μM. Studies with cloned taurine transporter from rat kidney cortex[41] or a mouse retinal library[42] expressed in Xenopus laevis oocytes has confirmed a high affinity transport system with a Km of 22.5 μM and 13.2 μM , respectively. To date, data describing the characteristics of active taurine accumulation in lens epithelial cells is not available nor has the impact of aldose sugars or their sugar alcohol counterparts on the taurine transport system been previously considered.

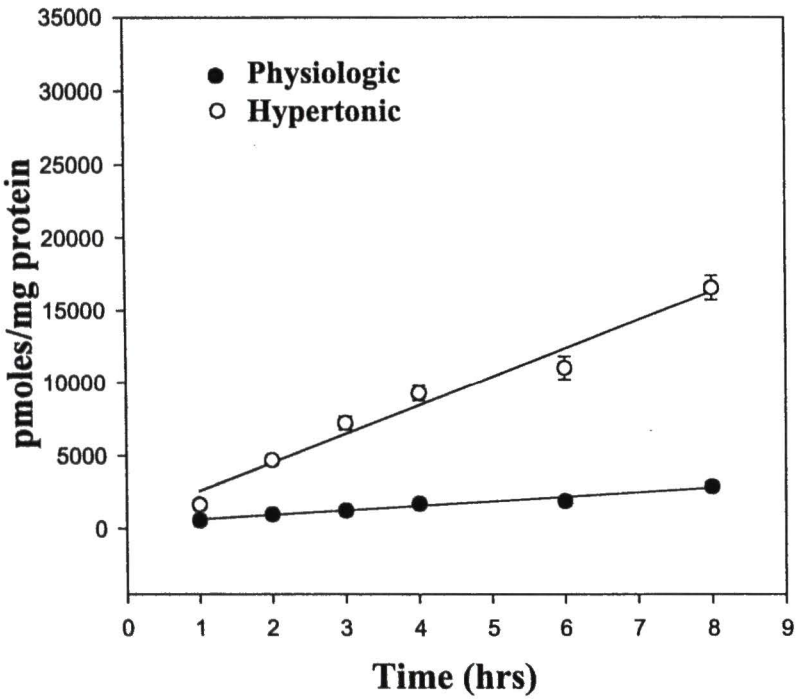
[^{3}H]-taurine uptake in cultured human lens epithelial cells was found to be unaffected by preincubation in 40 mmol/l galactose when compared to 40 mmol/l galactose with the coadministration of sorbinil (Fig. 2A), both curves being equivalent to physiological control. Contrariwise, the intracellular accumulation of galactitol did equate with an observed reduction in [^{3}H]-taurine uptake resulting from pre-exposure of cultured bovine

lens epithelial cells to 40 mmol/l galactose (Fig. 2B). The uptake curve for [^{3}H]-taurine in the presence of 40 mmol/l galactose and sorbinil was indistinguishable from physiological control (Fig. 2B). The observed inhibition of [^{3}H]-taurine accumulation could not be attributed to galactose as the uptake measurement was performed in galactose-free medium. Our kinetic measurement identified a high affinity taurine transport site. Based upon the velocity curve data, galactitol-induced inhibition of active taurine uptake for this high affinity transport site in BLECs is noncompetitive as there was a marked reduction in the V_{max} without significant alteration of the Km (Table 1).

Dixon plot analysis further confirmed that galactose does not interact with the high affinity taurine transport site with either human or bovine lens epithelial cells (Fig. 3A,B). These experiments were performed in the presence of extracellular galactose and sorbinil (thereby eliminating the possibility of galactitol formation and intracellular accumulation), so that they were designed to analyze the direct course of action of galactose on the taurine transport system. Similar experiments were also carried out with extracellular galactitol. Exogenous galactitol also did not interact with the taurine transport system as corroborated by Dixon plot analysis for either human (Fig 4A) and bovine (Fig. 4B) lens epithelial cells. These results were incompatible with the velocity curve data in figure 2B. It should be noted that experiments which utilize exogenous galactitol likely do not reveal the mechanism by which *intracellular* galactitol might influence taurine uptake. The duration of incubation of exogenous galactitol did not exceed three hours and galactitol does not readily cross cell membranes. Therefore, it is unlikely that exogenous galactitol could have accumulated inside the cell to a degree high enough to adversely affect the cytoplasmic side of the taurine transport system.

Collectively, these studies uncovered an incongruity between the *galactitol insensitivity* of the human taurine transport system (Figs. 2A) and the *galactitol sensitivity* of the bovine taurine transport system (Figs. 2B). That apparent discrepancy is likely explained by the higher rate of polyol formation and accumulation in the bovine lens epithelial cells as compared to the human lens epithelial cells (Table 2). The data is supportive of the fact that intracellular galactitol in excess of 325 nmol/mg protein likely has adverse affects on taurine transport. Whether this inhibition of taurine transport is the result of direct interaction with the taurine transport site or due to some secondary affect on cellular metabolism could not be determined from these data alone and further studies would appear to be warranted.

In conclusion, taurine transport was found to be unaffected by exposure to galactose. The taurine transport system appears to be hindered by intracellular galactitol only if a suitably high enough level of galactitol is amassed.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Hypertonic
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Time (hrs)
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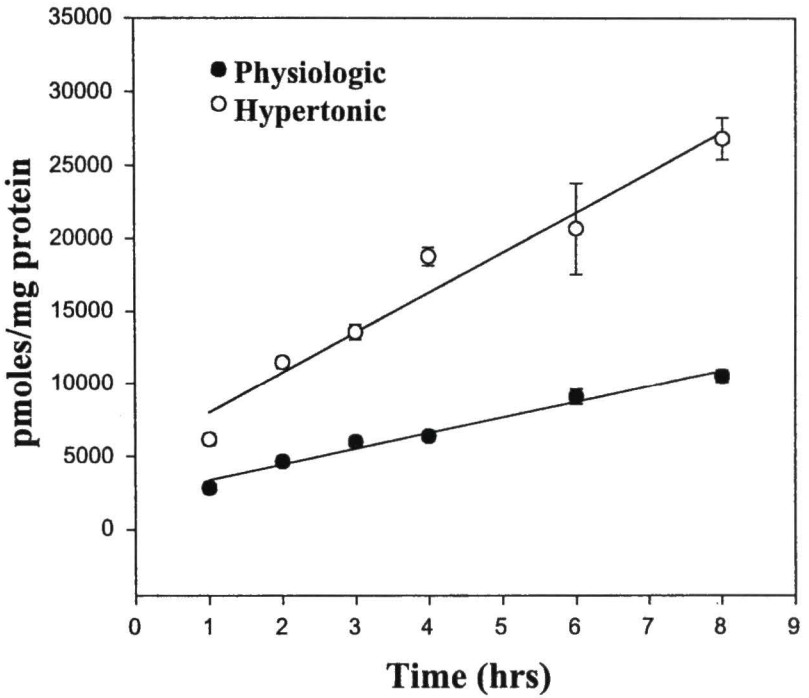
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30000
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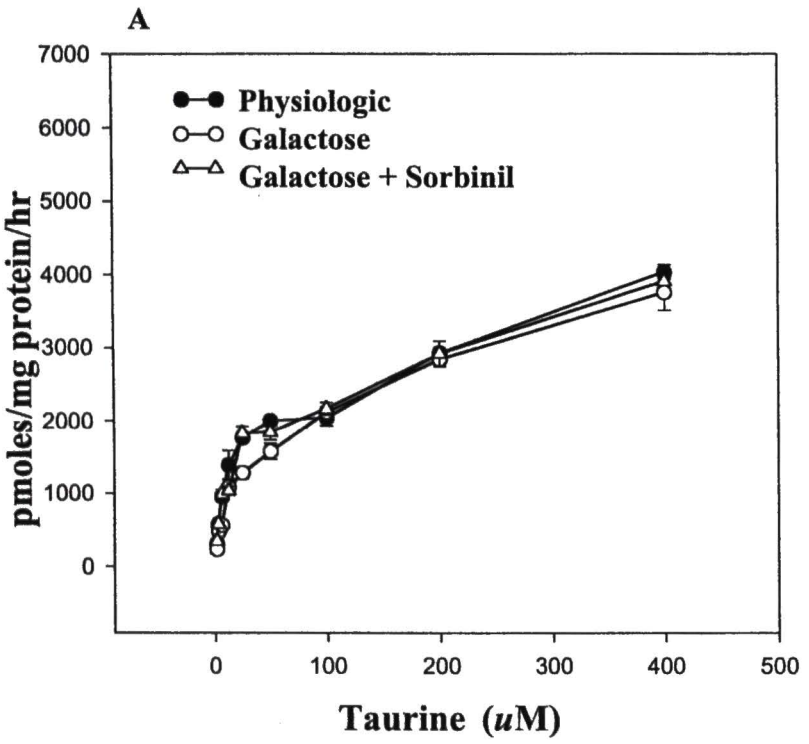
Figure 1. Time dependant [^{3}H]-taurine uptake. Human lens epithelium were incubated in 5% fetal bovine serum with medium A, while bovine lens epithelium were incubated in serum-free medium A. Both uptake media contained approximately 100 μmol/l [^{3}H]-taurine. Taurine uptake was determined by collecting triplicate flasks for each of the designated times. The data represent the mean ± standard errors. When the SEM is not visible, it is because the error bars are smaller than the symbol. Data points were plotted by linear regression. For human lens epithelium (**A**) the correlation coefficients for physiologic and hypertonic conditions were .95 and .91, respectively. For bovine lens epithelium (**B**) the correlation coefficients for physiologic and hypertonic conditions were .88 and .95, respectively.


A
Physiologic
Galactose
Galactose + Sorbinil
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4000
5000
6000
7000
0
100
200
300
400
500

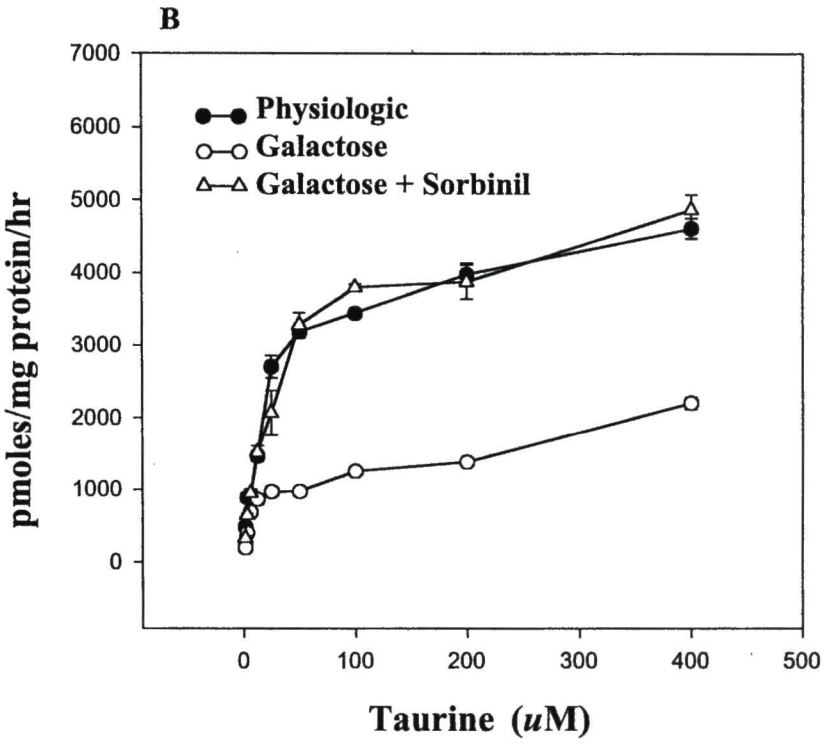
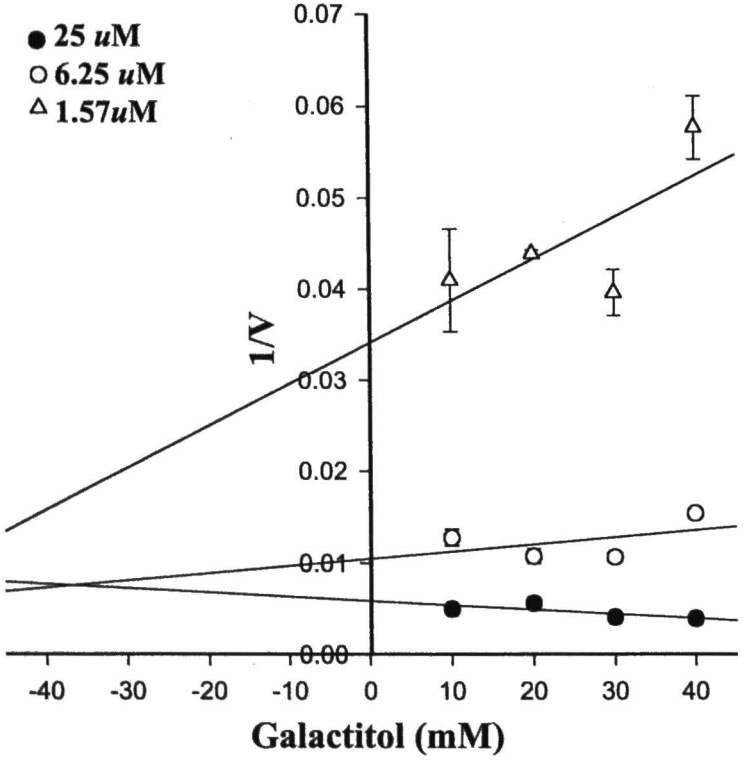

B
Physiologic
Galactose
Galactose + Sorbinil
pmoles/mg protein/hr
Taurine (uM)
0
1000
2000
3000
4000
5000
6000
7000
0
100
200
300
400
500

Figure 2. Effect of chronic galactose exposure and sorbinil on [^{3}H]-taurine uptake. Human (**A**) and bovine (**B**) lens epithelial cells were preincubated in 40 mmol/l galactose, 40 mmol/l galactose plus 0.1 mmol/l sorbinil or physiological medium (5.5 mmol/l glucose) for 20 hours and the experiment carried out as described in Materials and Methods. Note that galactose was not present during the 3-hr [^{3}H]-taurine uptake period performed over a concentration range of 1.5 to 400 μmol/l. Data represent triplicate determinations from individual flasks. Data points are means ± standard errors. *Significant from physiological medium using reciprocal data transformation and an independent T test comparison of the slope from a linear regression ($P<.001$).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○ 6.25 uM
△ 1.57uM
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
1/V
-40 -30 -20 -10 0 10 20 30 40
Galactitol (mM)

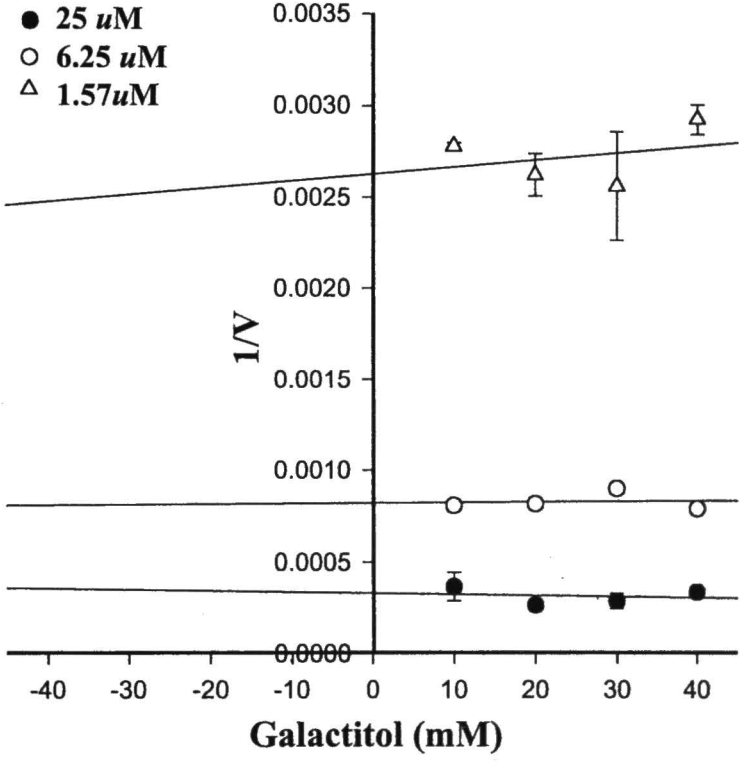
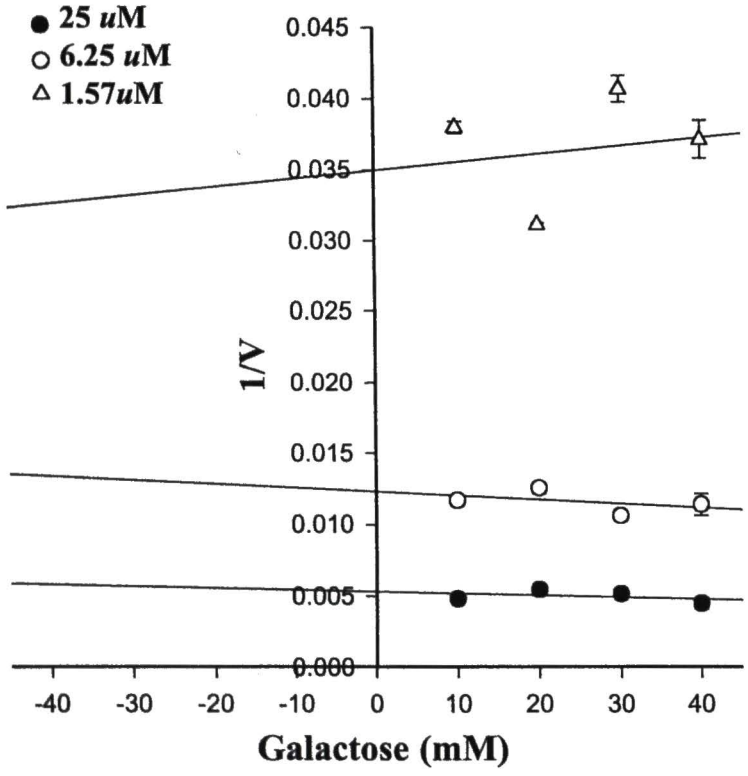

● 25 uM
○ 6.25 uM
△ 1.57uM
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
1/V
-40 -30 -20 -10 0 10 20 30 40
Galactitol (mM)

Figure 3. Dixon plot of acute D-galactose and sorbinil exposure on [^{3}H]-taurine uptake. Human (**A**) and bovine (**B**) lens epithelial cells were incubated in medium A containing 1.57 umol/l, 6.25 umol/l, and 25 umol/l taurine and a trace amount of [^{3}H]-taurine. Data points were plotted by linear regression and the correlation coefficients for the Dixon plot resulting from 1.57 umol/l, 6.25 umol/l, and 25 umol/l taurine were .60, .90, and .94 for (**A**) and .62,.95, and .94 for (**B**), respectively. The incubation mixtures for (**A**) and (**B**) also contained 10 mmol/l, 20 mmol/l, 30 mmol/l, or 40 mmol/l galactose plus 0.1 mmol/l sorbinil. Taurine uptake was determined after a 3-hr uptake period. Data points represent means ± standard errors of triplicate determinations from individual flasks.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6.25 uM
1.57uM
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0.040
0.035
0.030
0.025
0.020
0.015
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1/V
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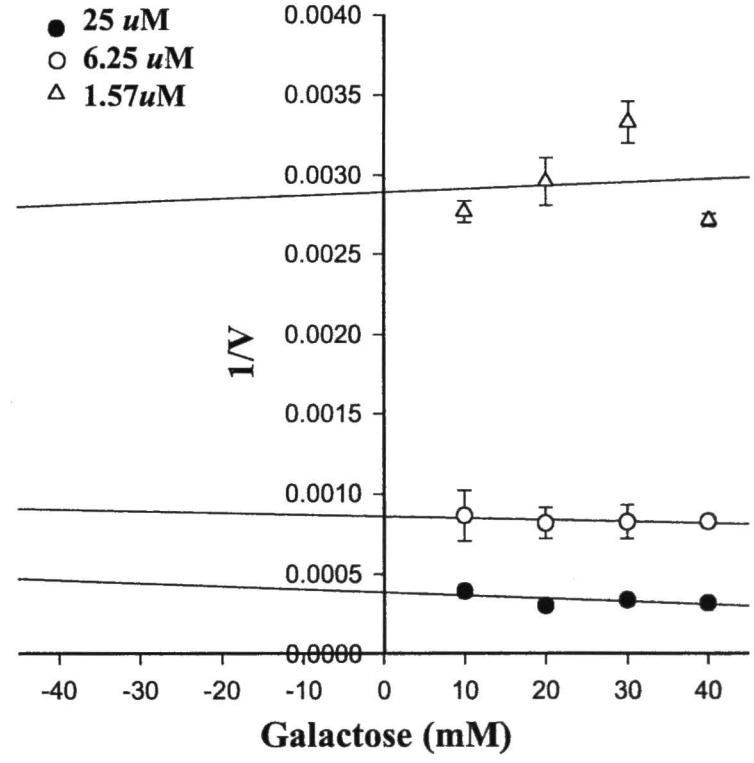
40
Galactose (mM)


25 uM
6.25 uM
1.57uM
0.0040
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0.0025
0.0020
0.0015
0.0010
0.0005
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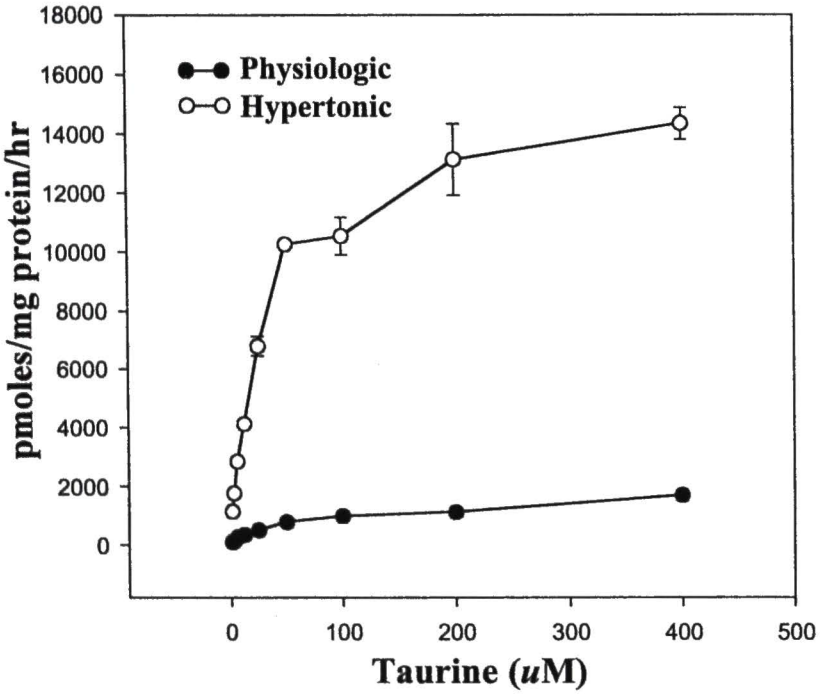
1/V
-40
-30
-20
-10
0
10
20
30
40
Galactose (mM)

Figure 4. Dixon plot of acute galactitol exposure on [^{3}H]-taurine uptake. Human (**A**) and bovine (**B**) lens epithelial cells were incubated in medium A containing 1.57 umol/l, 6.25 umol/l, and 25 umol/l taurine and a trace amount of [^{3}H]-taurine. Data points were plotted by linear regression and the correlation coefficients for the Dixon plot resulting from 1.57 umol/l, 6.25 umol/l, and 25 umol/l taurine for (**A**) were .65,.89, and .94 and (**B**) .65,.93, and .94, respectively. The incubation medium of (**A**) and (**B**) also contained 10 mmol/l, 20 mmol/l, 30 mmol/l, or 40 mmol/l galactitol. Taurine uptake was determined after a 3-hr uptake period. Data points represent means ± standard errors of triplicate determinations from individual flasks.


Physiologic
Hypertonic
pmoles/mg protein/hr
Taurine (uM)
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16000
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12000
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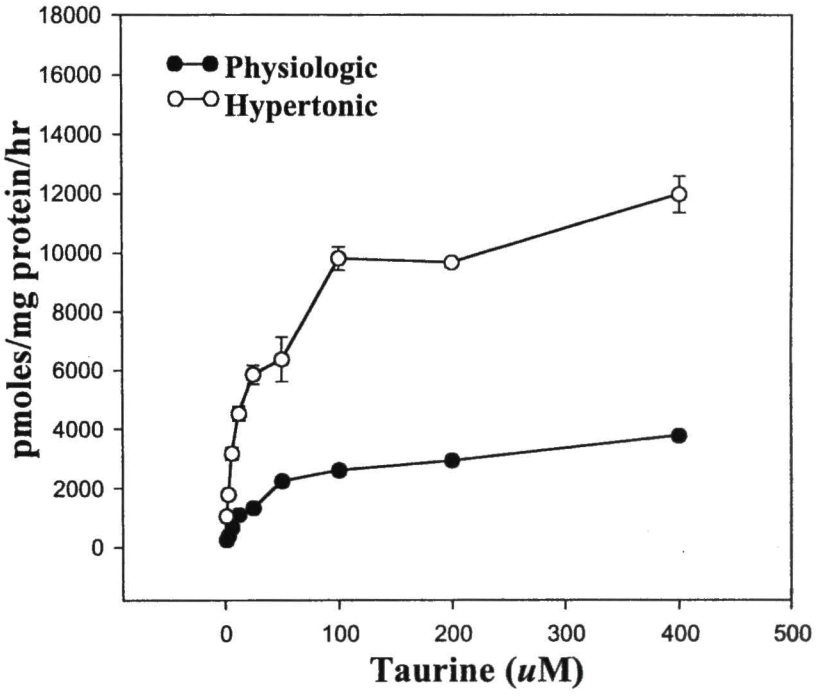
Physiologic
Hypertonic
pmoles/mg protein/hr
Taurine (uM)
18000
16000
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Figure 5. Effect of hypertonicity on [^{3}H]-taurine uptake. Human (**A**) and bovine (**B**) lens epithelial cells maintained in physiological medium or physiological medium adjusted with 116 mmol/l sodium chloride for 20 hours and subsequently treated as described in Materials and Methods. Data points represent mean ± standard errors taken from triplicate determinations of individual flasks. The hypertonicity curves were significant from physiological curves using reciprocal data transformation and an independent T test comparison of the slope from a linear regression ($P<.001$).

Table 1. Kinetic Parameters of the Taurine Transporter in Human and Bovine LensEpithelial Cells in Physiological and Galactose-Supplemented Medium

Conditions	Km		Vmax	
	Human	**Bovine**	**Human**	**Bovine**
Physiological (5.5 mmol glucose)	7.2 ± 1.8	21.4 ± 1.7	1891 ± 204	4546 ± 98
Galactose (40mmol/l)	7.7 ± 3.1	17.0 ± 2.9	1510 ± 297	1737 ± 73*
Galactose + Sorbinil (0.1 mmol/l)	7.1 ± 2.0	28.5 ± 3.2	1926 ± 174	4884 ± 153

Values are mean ± SE of three individual experiments. The unit for Km and Vmax are μM and pmol/mg protein /hr respectively. ("*" denotes significant difference, $p<.05$).

Table 2. Intracellular galactitol content in Human and Bovine Lens Epithelial Cells

Conditions	Galactitol Content	
	Human	**Bovine**
Physiological (5.5 mmol glucose)	ND	ND
Galactose (40mmol/l)	60 ± 1.6	334 ± 7.0

Values were acquired by anion exchange chromotography an d are means ± SE expressed as nmom/mg protein. Data points represent duplicate determinations from triplicate flasks. ND: non-detectible

Table 3. Kinetic Parameters of the Taurine Transporter in Human and Bovine Lens Epithelial Cells in hypertonic medium

Conditions	Km		V_{max}	
	HLE-B3	**Bovine**	**HLE-B3**	**Bovine**
Physiological(5.5 mmol glucose)	19.1± 3.4	21.4 ± 3.5	1891 ± 204	2757 ± 424
Hypertonic(232 mmol NaCl)	25.5 ± 7.7	17.0 ± 2.9	14633 ± 908*	9999 ± 461*

Values are means ± SE. The unit for Km and Vmax are uM and pmol/mg protein/hr respectively. ("*" denotes significant difference, $p<.05$).

CHAPTER III

Conclusion

This study shows the effect of three extreme environments on two different species of cultured lens epithelial cells. Cells were placed under chronic galactose conditions. We were then able to observe the effect of galactose and its corresponding sugar alcohol, galactitol. Cells were also placed in hypertonic, high salt, conditions in order to provide support to prior findings within the lab that the taurine transporter upregulates in such a condition. To our knowledge, this is this first time uptake kinetic taurine studies have been performed on lens cells.

The taurine transporter is known to be dependent on Na+ and Cl- on for intracellular accumulation. The effect of hypertonicity on MDCK cells was reported by Uchida (1991) as drastically increasing taurine transport while leaving the transport affinity unchanged.[23] The findings here in two other cell lines, human and bovine lens cells (Fig. 2), support the observations in this prior study. Because the transport (Vmax) is increased without a concurrent change in the affinity (Km), it is inferred that this is due to an increase in operational transporters and not an increased taurine affinity by this transporter.

There was also an increase in taurine accumulation over time. Through an 8 hour uptake period, a continued increase in accumulation was observed in both cell lines, while maintaining linearity (Fig.1). This experiment was not taken to further time points as the cells soon became too stressed in this chronic hypertonic condition. From these experiments it would appear that in both of these lens cell lines, as seen in cells of other tissues, taurine is a major role player in their response to osmotic stress.

The second part of this study deals with the effect of galactose and galactitol on taurine transport. In the bovine lens cell culture, the results were similar to those found in the same cells tested for myo-inositol uptake.[9] Incubation with high galactose conditions significantly decreased the taurine accumulation (Fig. 3B). The significant decrease was due to a decrease in Vmax with little, if any, change in Km. Results such as these are highly indicative of noncompetitive inhibition. The normalization of the velocity curve upon the addition of sorbitol, the aldose reductase inhibitor, implies, as in the previous study, that the sugar alcohol accumulation within the cell impedes the accumulation of an osmolyte such as taurine. When the same principles were applied to the human lens cells, however, there was no significant decrease in Vmax for those cells incubated in chronic galactose conditions (Fig. 3A). When incubated with galactose plus sorbinil there was no change, as would be expected from previous experiments. It appears as though the intracellular accumulation of galactitol has no effect on the human cell. There was also no significant difference from the physiologic state was recorded for either or the experimental conditions for Km. This would appear to point out obvious difference in the uptake mechanism between these two lens cell species which could be due to a

higher aldose reductase activity in the bovine lens cells, thus allowing the cell to convert more galactose to galactitol within the cell.

Analysis of both species through Dixon plots allows us to determine the underlying difference for the discrepancy between species. Dixon plot analysis with galactose were performed with sorbinil. This is to ensure that we are testing the cells reaction to only galactose by preventing galactitol accumulation. The extracellular galactitol, however, does not accumulate within the cell. The cell membrane presents an impervious structure to the sugar alcohol. Hence, the galactitol is only effecting the external portion of the transporter. Bovine lens cells did not show any sort of inhibition in its exposure to the high galactitol environment (Fig. 5B). This, as stated before, shows a different behavior than the accumulation of the osmolyte myo-inositol in the same cell line under the same conditions. In that study, the resultant exposure to galactose was similar to the outcome experienced here (Fig. 4B). This is a contradiction however, with the earlier findings within this paper. With the accumulation of galactitol in the velocity curve experiment, there was an obvious inhibition in taurine uptake. It must once again be stated, however that this experiment is only testing the external portion on the transporter. Thus, it appears as though there must be an internal accumulation of galactitol for inhibition to take place.

The human lens cells exposure to both the high galactitol and high galactose environments gave a more expected result. As in the velocity curve, no inhibition of taurine uptake was observed. Neither the intracellular nor extracellular (Fig. 5A) presence of galactitol produced an effect. The same was true for galactose alone (Fig. 4A).

If intracellular polyol accumulation is the main constituent in diabetic cataracts, it would appear as though, much more convincingly at least, that in the human lens cell taurine flux is not a major player. What is causing the inhibition in the bovine lens cells is most likely due to the very high amount of galactitol that has been accumulated, most likely due to higher aldose reductase activity. Taurine does, however, appear to be a major osmolyte for both species of lens cells. A high salt environment appeared to increase taurine accumulation both over time and in a saturation type fashion depicting that a transporter is at work.

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