REGULATION OF TAURINE TRANSPORTER ACTIVITY IN CULTURED RAT RETINAL GANGLION CELLS AND RAT RETINAL MÜLLER CELLS

Laila A. Eissa [1*], Sylvia B. Smith[2],Amira A. El-Sherbeny[3]

يعتبر الاعتلال الشبكي من أكثر المضاعفات الشائعة لمرض السكر. ومن المعتقد أن الحمض الأميني تورين (taurine) يلعب دوراً واقياً مضاداً للأكسدة في الاعتلال الشبكي وذلك عبر اقتناص الأصناف المتفاعلة. ومن غير المعروف بعد ما إذا كان تمثيل مادة تورين في الخلايا الشبكية يتغير أثناء مرض السكر أم لا. ولهذا صممت هذه الدراسة لبحث التغيرات في نقل مادة تورين في زراعات خلايا موللر الشبكية للجرذان وخلال العقد العصبية الشبكية للجرذان في الحالات المصاحبة لمرض السكر. وقد تمت عملية امتصاص مادة تورين بكثرة بواسطة خلايا موللر الشبكية للجرذان والعقد العصبية الشبكية لها تحت ظروف السكر الطبيعية. كما تم النقل الفاعل لمادة تورين إلى خلايا موللر وإلى خلايا العقد العصبية الشبكية بطريقة معتمدة على كل من أيون الصوديوم وأيون الكلور. وقد ازداد امتصاص مادة تورين في كلا النوعين من الخلايا بعد الحضن مع تركيز عال من مادة جلوكوز. ويمكن أن يعزى هذا التأثير إلى الزيادة في الأسمولية (osmolarity). ولأن لمادة أكسيد النيتريك علاقة بحدوث مرض السكر، فقد قمنا بتقدير نشاط نقل مادة تورين في خلايا موللر الشبكية المزروعة وخلايا العقد العصبية الشبكية للجرذان في وجود مانحات أكسيد النيتريك وهما SNAP, SIN-1. وقد ارتفع امتصاص أو تمثيل مادة تورين فوق القيم الشاهدة (الكنترول) بعد فترة حضانة مقدارها 24 ساعة مع تركيز منخفض من مانحي أكسيد النيتريك. وأخيراً ، فقد تم بحث مدى قدرة الجلوتامات السامة عصبياً على تغيير نشاط نقل مادة تورين في كلا النوعين من الخلايا. وقد ازداد امتصاص مادة تورين معنوياً في خلايا العقد العصبية الشبكية عندما تم حضنة فقط مع تركيز كبير من مادة جلوتامات. وقد دلت البيانات على أن ناقل مادة تورين موجود في خلايا العقد العصبية الشبكية المزروعة للجرذان وفي خلايا موللر ويتم تنظيمها بواسطة فرط الأسمولية. وتتعلق هذه البيانات بأمراض مثل البول السكري وانحلال الخلايا العصبية حيث يتغير حجم الخلايا الشبكية بشكل كبير.

Diabetic retinopathy is one of the most common complications of diabetes. The amino acid taurine is believed to play an antioxidant protective role in diabetic retinopathy through the scavenging of the reactive species. It is not well established whether taurine uptake is altered in retinal cells during diabetic conditions. Thus, the present study was designed to investigate the changes in taurine transport in cultures of rat retinal Müller cells and rat retinal ganglion cells under conditions associated with diabetes. Taurine was abundantly up taken by rat retinal Müller cells and rat retinal ganglion cells under normal glycemic condition. Taurine was actively transported to rat Müller cells and rat retinal ganglion cells in a Na^+ and Cl^- dependent manner. Taurine uptake further significantly elevated in both types of cells after the incubation with high glucose concentration. This effect could be attributed to the increase in osmolarity. Because nitric oxide (NO) is a molecule implicated in the pathogenesis of diabetes, we also determined the activity of the taurine transporter in cultured rat retinal Müller cells and rat retinal ganglion cells in the presence of the NO donors, SIN-1 and SNAP. Taurine uptake was elevated above control values after 24-h incubation with low concentration of NO donors. We finally investigated the

[1]Department of Biochemistry, College of Pharmacy, Mansoura University, Mansoura 35516, Egypt. Departments of [2] Cellular Biology and Anatomy, and [3]Ophthalmology, Medical College of Georgia, Augusta, Georgia 30912, USA,

*To whom correspondence should be addressed.
E-mail lailaeissa2002@yahoo.com

ability of neurotoxic glutamate to change taurine transporter activity in both types of cells. Uptake of taurine was significantly increased in rat retinal ganglion cells when only incubated with high concentration of glutamate. Our data provide evidence that taurine transporter is present in cultured rat retinal ganglion and Müller cells and is regulated by hyperosmolarity. The data are relevant to diseases such as diabetes and neuronal degeneration where retinal cell volume may dramatically change.

Key words: Taurine transporter, rat retinal ganglion cells and Muller cells, NO donors, glutamate.

Introduction

TAURINE, an aminosulfonic acid, is the most common free amino acid in the retina (1). A high concentration of taurine is important for maintenance of the structural and functional integrity of the retina (2,3). The precise physiological role of taurine in the retina is uncertain, although it has been suggested to function in calcium modulation (3-5), osmoregulation (6-8), protein stabilization and stress response (9). Taurine has been proposed to function as an osmolyte (9, 10) because the intracellular concentration of taurine in retina is 20-40 mM. Taurine has been also shown to have antioxidant property because it protects rod outer segment membranes from ion as a consequence of membrane lipid peroxidation (11). Recent study has shown that taurine, in combination with retinol, protects lipids from oxidative damage and may play a key role in protecting retinal pigment epithelial (RPE) lipids during exposure to cyclic light (12).

In the retina, the concentration of taurine is highest in photoreceptor and RPE cells (13, 14). Functional studies suggest that a transporter for taurine is present on the apical membrane of RPE (15) and in cultured human RPE cells (16). The taurine transporter in various cell types, including RPE cells, is regulated by signal transduction pathways (17, 18), hypertonicity (19), and extracellular taurine levels (20).

Recently, the effect of diabetes on taurine transporter activity was investigated. Previous study has shown that high glucose levels rapidly and specifically decreased the activity and mRNA levels of the transporter in cultured RPE cells (21). In contrast, in vivo studies of RPE from diabetic rats showed that uptake of taurine was elevated, rather than decreased, suggesting that diabetes stimulates the activity of the taurine transporter (22).

Nitric oxide (NO) is implicated in the pathogenic complications of diabetic retinopathy (23). Circulating levels of NO are elevated in diabetes mellitus (5, 24). Yilmaz and co-workers (25) reported a fivefold elevation of NO in the vitreous of patients with proliferative diabetic retinopathy compared with nondiabetic control patients. There is evidence that NOS activity is increased in retinas of diabetic rats (26).

The neurotic compounds such as glutamate also significantly increased as a result of diabetic complications (9). Given that NO levels are increased in diabetic retinopathy and that the diabetic condition may affect taurine transport, we were interested in determining the effects of NO on taurine transport in vitro. In the present study, we tested the change in taurine transport activity in cultured rat retinal Müller cells and rat retinal ganglion cells under normal and hyperglycemic conditions. We also determined the effects of various NO donors and neurotoxic glutamate on the activity of the taurine transporter in both types of cells.

Materials and Methods

Chemicals:

D-(+)-glucose, calcium chloride, magnesium sulfate, sodium chloride, 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES), Trizma base, potassium chloride, D-Mannitol, L-glutamic acid, succinyl concanavalin-A (SconA), S-Nitroso-N-acetyl-penicillamine (SNAP), and Folin and Ciocalteu's reagent were purchased from Sigma Chemical Corp. (St. Louis, MO). 3-Morpholinosydnonimine (SIN-1) was purchased from Research Biochemical's International (Natick, MA).

Radiolabeled compounds:

[1, 2-^{3}H]-taurine (specific radioactivity, 21.0 Ci/mmol), L-[^{3}H(N)]-carnitine (specific radioactivity, 65 Ci/mmol), L-[carboxyl-^{14}C]-ascorbic acid (specific radioactivity, 17.0 mCi/mmol), and L-[2, 3, 4, 5-^{3}H]-arginine monohydrochloride (specific radioactivity, 55 Ci/mmol), were purchased from Amersham Pharmacia (Piscataway, NJ). L-[3, 4-^{3}H]-

glutamine (specific radioactivity, 40Ci/mmol), and L-[2, 3-^{3}H]-glutamate (specific radioactivity, 24 Ci/mmol) were purchased from NEN Research Products (Boston, MA). [3', 5', 7, 9-^{3}H]-N^{5}-methyltetrahydrofolate (specific radioactivity, 30 Ci/mmol) was from Moravia Biochemical's (Brea, CA).

Cell lines:

In this study we used two types of cell lines, a rat Müller cell line (RMC-1) and a rat ganglion cell line (RGC-5).

Cell culture:

Cell culture supplies, which include Dulbecco's Modified Eagle Medium: nutrient mixture F12 (DMEM/F12), RPMI 1640 media, 100U/ml penicillin, 100µg/ml streptomycin, and 0.05% (w/v) trypsin, were purchased from Life Technologies (Gaithersburg, MD). Fetal bovine serum (FBS) was purchased from Atlanta Biological (Norcross, GA). Mouse laminin was purchased from Collaborative Biomedical Products (Bedford, MA). Corning 24-well plates and 75cm^{2} flasks were purchased from Fisher Scientific (Norcross, GA).

Method

Cell culture:

RMC-1 cells, a rat retinal Müller cell line, and RGC-5 cells, a rat retinal ganglion cell line, were used for these experiments. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were grown in 75 cm^{2} flasks and maintained with Dulbecco's modified Eagle medium (DMEM): nutrient mixture F12, supplemented with 10% FBS, 100U/ml penicillin and 100µg/ml streptomycin. After becoming confluent, cultures were passaged by dissociation in 0.05% (w/v) trypsin in phosphate-buffered saline (PBS). After trypsinization, RMC-1 and RGC-5 cells were seeded onto 24-well plates, which were coated with 5 µg/cm^{2} mouse laminin in experiments with ganglion cells and in some of the experiments of Müller cells. Laminin promotes growth, adhesion and protein synthesis in cells. RGCs were kept for 24 hours in serum free medium, and then maintained for 6-7 days with DMEM: F12 supplemented with 1% FBS and 100 U/ml penicillin and 100 µg/ml streptomycin. The serum content of the medium was decreased from 10% to 1% to promote differentiation of the cells. Succinyl concanavalin A was added to the 1% medium to facilitate differentiation. RMCs were seeded and grown on 24 well plates directly and most of experiments done with these cells were done when they become confluent, which was typically on the third day following seeding. There was one set of experiments analyzing effects of glucose where cells were grown and maintained on laminin coated 24 well plates in medium containing 1% serum for 3-4 days. In this set of experiments we were trying to look for the effect of low and high levels of glucose on the uptake of taurine as in this case cells needed to be grown for a longer period of time.

Uptake assays

To determine the functional activity of the taurine transporter, uptake experiments were used. For this assay the culture medium was removed from the cells and they were washed once with warm NaCl uptake buffer. The composition of this buffer was 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethan-sulfonic acid (HEPES)/Tris, 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5mM glucose, pH 7.5. In some experiments, N-methyl-D-glutamate chloride was substituted for NaCl to establish the NaCl-dependency of taurine uptake. Uptake was initiated by adding 250 µl of uptake buffer containing radio labeled substrates, usually (0.5 µCi/well). Uptake measurements were perfor-med with a 15 min. incubation at 37°C. The uptake was terminated by removal of the medium by aspiration followed by two washes with ice-cold uptake buffer without the radio labeled substrates. The cells were solubilized in 0.5 ml of 1% sodium dodecylsulfate in 0.2N NaOH and transferred to scintillation vials for quantitation of radioactivity.

Substrate specificity experiment:

To determine the functional activity of taurine transporter, a substrate specificity experiment was performed using uptake assays for different compounds including taurine, glutamine, carnitine, arginine, glutamate, methyltetrahydrofolate, and ascorbate. Müller and ganglion cells were seeded onto 24-well plates and the 15 minutes uptake of each compound was measured. The specific activity of all compounds was (0.5 µCi/ well) radioactivity. The concentration of each compound would be different as it depends on the specific activity of each substance. Thus a standard measurement of the radioactivity of a 25 µl of each compound was

measured. To confirm the Na^+ and Cl^- dependency of the taurine transporter, cells were seeded and cultured as described above. At time of experiment cells were incubated with radio labeled taurine (0.5 µCi/well) for 15 minutes in presence of NaCl buffer, in absence of Na^+ but in presence of Cl^- (N-methyl-D-glutamate chloride) or in presence of Na^+ but the absence of Cl^- (Na gluconate). The inhibition of taurine uptake by (2.5 mM) ß-alanine, a known competitive inhibitor of taurine uptake (Vinnakota *et al*. 1997), was also assessed.

Incubation with high glucose:

For experiments in which cells were grown in high glucose, RPMI medium was used. RPMI containing no glucose is commercially available and thus permits addition of whatever glucose amount is required to achieve the desired concentration. Cells were grown for about one week in RPMI media. In this set of experiments both Müller and ganglion cells were seeded on laminin coated 24 well plates for 3 and 6 days respectively. They were cultured during this period of time in medium containing 5 mM glucose. At time of the incubation, the glucose level was adjusted to either low glucose concentration 5 mM as a control for the normal physiological condition, with 5 mM glucose plus 35 mM mannitol as a control for osmolality, or with high glucose 40 mM for 24 hours.

Incubation with nitric oxide (NO) donors:

Müller cells were seeded and grown in DMEM/F12 media as before until become confluent. In case of ganglion cells, they were grown and differentiated in 1% serum DMEM/F12 media with SconA. At time of incubation, cells were treated with different concentrations of either 3-morpholinosydnonimine (SIN-1) or S-nitroso-N-acetyl-penicillamine (SNAP) dissolved in dimethyl sulfoxide (DMSO). The different concentrations used were 10µM, 100µM, and 1mM, which were prepared from a 100 mM stock solution of SIN-1 and SNAP in DMSO. Control cells were exposed to DMSO of the same concentration. Uptake of [^{3}H] taurine was performed using a concentration of 80 nM (specific activity of 0.5 µCi /well) for 15 minutes.

Glutamate incubation:

Cells were seeded from a half confluent flask into 24 well plates and cultured until become confluent in case of Müller cells and differentiated in case of ganglion cells. Cells were treated with different concentrations of L-glutamate that was dissolved in media and filtered with a sterile 25mm syringe filter to avoid contamination. These different concentrations were prepared as serial dilutions of (10 µM, 100 µM, 1 mM) from a stock solution of 100 mM and incubated with the cells for 24 hours. For control experiments glutamate was omitted from the medium. Uptake of [^{3}H] taurine (80 nM) was performed by adding 250 µl of buffer containing 0.5 µCi of this compound for a 15 min. incubation at 37°C.

Protein assay:

For each uptake experiment, one well of the 24-well plate is reserved for the measurement of protein content of cells. This content was used to calculate the data, which is expressed as femto-mol/mg protein. Analysis of protein was performed according to the method of Lowry (19) To generate a standard, 5 ml of 0.2% Na_2CO_3 in 0.1 N NaOH solution, 50 µl of 2% tartarate, and 50 µl of 1% $CuSO_4$ were added to 1 ml de-ionized water. For protein measurement in cells, a 100 µl and 200 µl aliquot of the sample are added to the same components brought to a final volume of 1 ml with H_2O. 500 µl of 1 N Folin Ciocalteu's reagent is added for 30 min. prior to measuring the absorbance spectrophotometrically at wave length 660 nm.

Data Analysis:

Data was calculated as fmol/mg protein by multiplying the radioactivity counts by a factor including the protein assay, the amount taken of standard and its radioactivity count. Radioactivity count (counts/min.)* Factor,

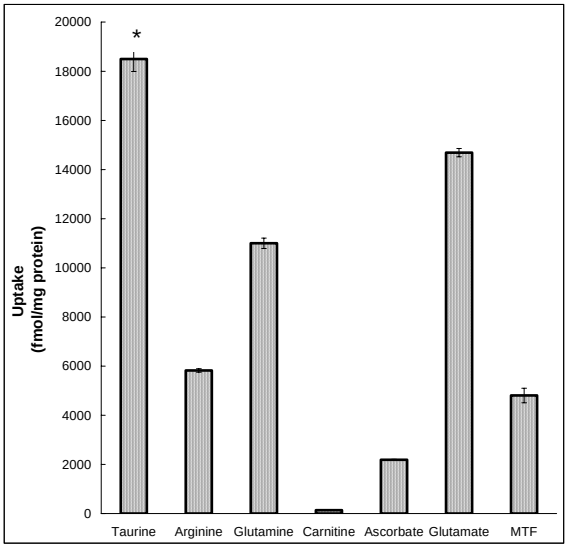
This factor is calculated as following: (25µl* standard count)/ protein (mg). Each uptake experiment was performed in duplicate or triplicate and was repeated 2-4 times. Data analysis (analysis of variance) was performed using the NCSS statistical software package ($p<0.05$ was considered significant). Data are presented as mean values $\pm$ S.E.

Results

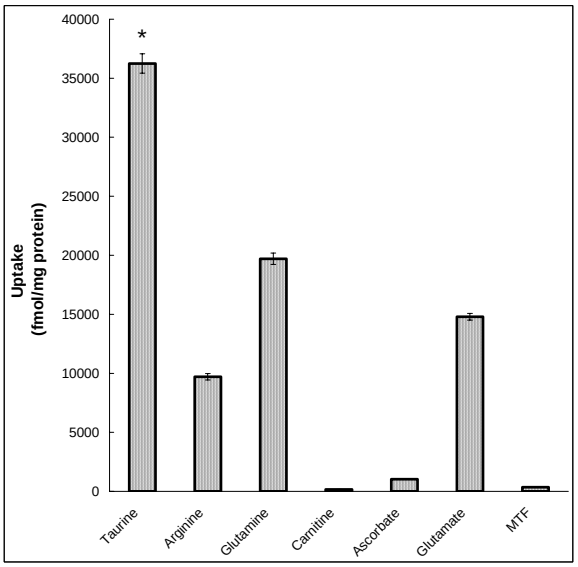
The present study focused on the change in taurine transporter activity in both rat retinal Müller cells and ganglion cells under hyperglycemic

condition and determined role of NO in the regulation of the taurine transporter in these cultured cells.

We initially tested the uptake of rat retinal Müller cells and rat retinal ganglion cells to various radiolabel led compounds such as arginine (55 Ci/mmol), ascorbate (17.0 mCi/mmol), carnitine (65 Ci/mmol), glutamate (24 Ci/mmol), glutamine (40Ci/mmol), methyltetrahydrofolate (30 Ci/mmol), and taurine (21.0 Ci/mmol). As shown in **Figure 1** of the compounds tested, taurine was taken up most avidly by both cultured rat retinal Müller cells and rat retinal ganglion cells ($P < 0.05$). To determine ion dependency of the taurine transporter in both types of tested cells, the uptake of [3H] taurine was measured in the presence of NaCl in the absence of Na^+ but in the presence of Cl (N-methyl-D-glucose chloride) or in the presence of Na^+ but the absence of Cl (sodium gluconate). The inhibition of taurine uptake by -alanine, a known competitive inhibitor of taurine uptake, was also assessed in these cells. As shown in **Figure 2**, taurine uptake was observed only in the presence of NaCl in both types of cells. In the absence of Na^+ or Cl^-, the uptake was reduced. These data suggest that the uptake of taurine in rat retinal Müller cells and rat retinal ganglion cells is NaCl dependent. **Figure 3**. shows the effect of high glucose concentration on tuarine transport in both types of cells. Incubation of rat retinal Müller cells and rat retinal ganglion cells with high concentration of glucose (45 mM) significantly increased [3H] taurine uptake. These effects seem to be due to an osmolar effect as incubation of both types of cells with low concentration of glucose + mannitol showed no significance difference when compared with high glucose concentration. **Figure 4**. shows the effect of the NO donors SIN-1 and SNAP on the uptake of [^{3}H] taurine by the taurine transporter in rat retinal Müller cells and rat retinal ganglion cells. Both NO donors, at low concentration and when incubated with cells for 24 h, stimulated uptake process, while high concentration of SIN or SNAP tended to kill both types of cells. SIN-1 was a more potent stimulator of taurine uptake than SNAP. **Figure 5**. Shows the effect of excitotoxic glutamate on the uptake of [^{3}H] taurine in both rat retinal Müller cells and rat retinal ganglion cells. Only incubation of rat retinal ganglion cells with high concentration of excitotoxic glutamate (1 mM) significantly increased [^{3}H] taurine uptake suggesting the importance of taurine as antioxidant in antagonizing neurotoxic effects of glutamate in rat retinal ganglion cells.

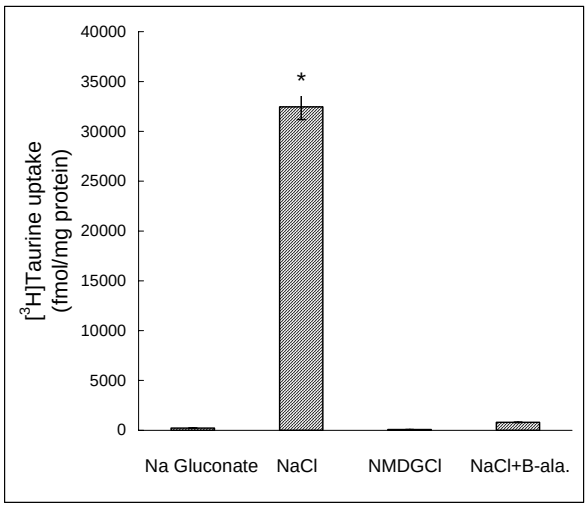


A: Ganglion cells

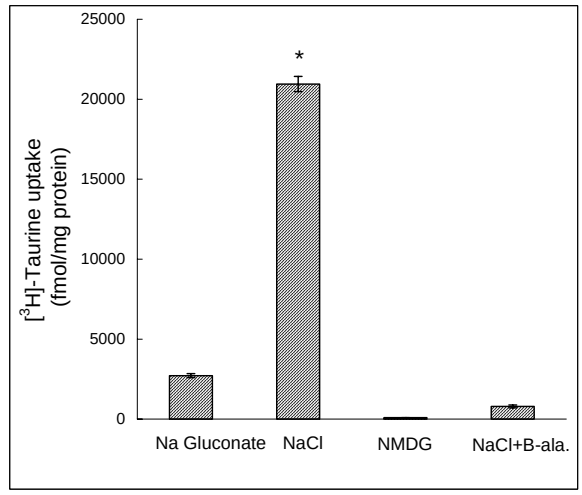


B: Müller cells

Figure 1. Substrate specificity of the transport of various radiolabelled compounds in the cultured rat retinal ganglion cells (A) and rat retinal Müller cells (B). Values represent mean ± S.E of two experiments performed in quadruplicate. * means significant when compared with carnitine, ascorbate and methyltetrahydrofolate (MTF) and arginine amino acids ($P < 0.05$).

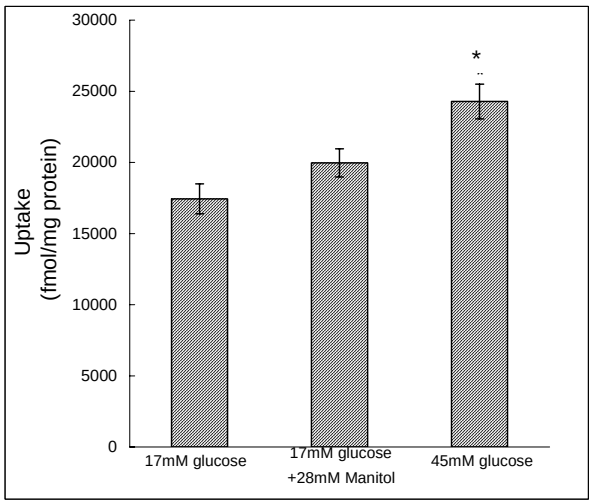


A: Ganglion cells

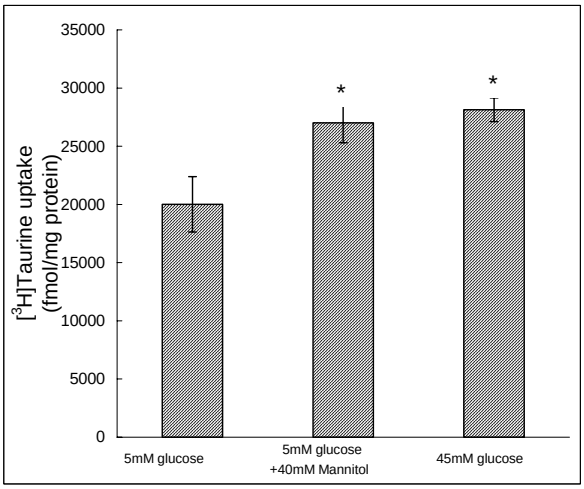


B: Müller cells

Figure 2. Determination of the ion dependence of the transport process mediated by the taurine transporter in the cultured rat retinal ganglion cells (A) and rat retinal Müller cells (B) using [^{3}H] taurine. Transport of taurine was measured in uptake medium that contained sodium chloride, sodium gluconate, NMDG chloride, or sodium chloride with ß-alanine. Values represent mean ± S.E of two experiments performed in quadruplicate. * significant when compared with the uptake in cells cultured in Na^+ only or Cl^- only containing media ($P < 0.05$).

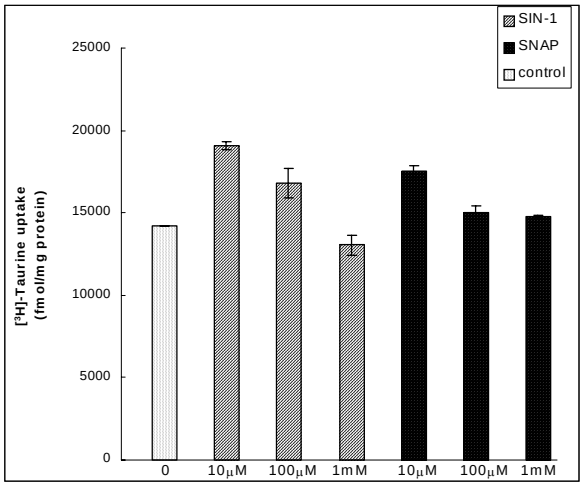


A: Ganglion cells

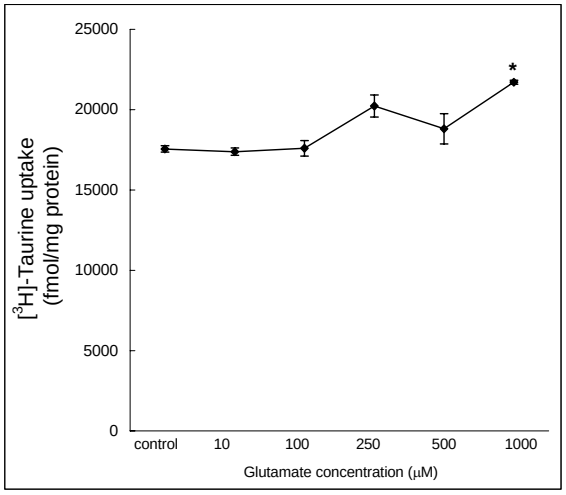


B: Müller cells

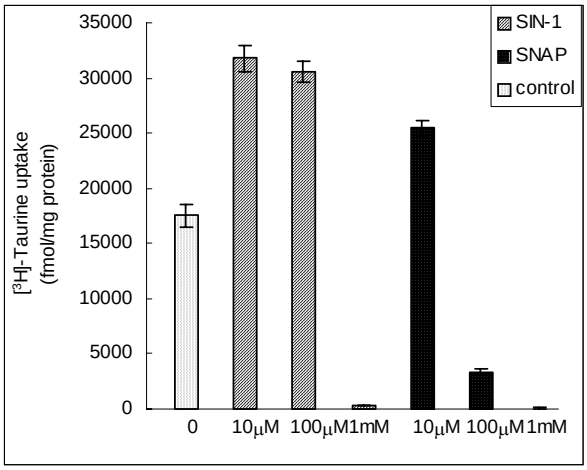
Figure 3. Effect of a high concentration of glucose on taurine uptake in the cultured rat retinal ganglion cells (A) and rat retinal Müller cells (B). Transport of [^{3}H] taurine was measured in uptake medium that contained low and high glucose concentrations. Values represent mean ± S.E of two experiments performed in quadruplicate. * significant when compared with the transport in medium that contained 5 mM glucose containing media ($P < 0.05$).



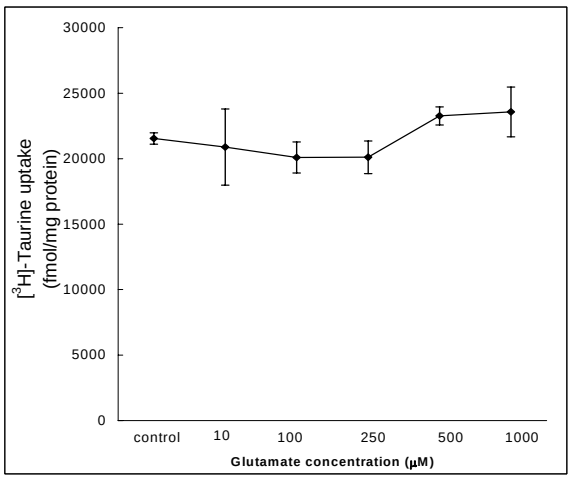
A: Ganglion cells



A: Ganglion cells



B: Müller cells



B: Müller cells

Figure 4. Effect of SIN-1 and SNAP, nitric oxide donors, on the uptake of taurine in the cultured rat retinal ganglion cells (A) and rat retinal Müller cells (B). Uptake of [^{3}H] taurine was measured in the absence (control) or in the presence of SIN-1 or SNAP at graded concentrations. Values represent mean ± S.E of two experiments performed in quadruplicate. * significant when compared with the control ($P < 0.05$).

Figure 5. Effect of excitotoxic glutamate on the transport of taurine in the cultured rat retinal ganglion cells (A) and retinal Müller cells (B). Uptake of [^{3}H] taurine was measured in the absence (control) or in the presence of glutamate at a concentration of 10, 100, 250, 500 or 1000 μM. Values represent mean ± S.E of two experiments performed in quadruplicate.

Discussion

One of the major phenomenon that associates the diabetic retinopathy is the disturbance in some amino acids transport including taurine. Recent studies have shown that taurine presents in high concentration in the retina and it plays an essential role in maintaining the structural and functional integrity of the retina (9). The present study was designed to test the change in taurine transport in rat retinal Müller cells and rat retinal ganglion cells under the condition of high glucose concentration. We also investigated the effect of NO and glutamate on the transport of taurine by cultured rat retinal Müller cells and rat retinal ganglion cells.

We sought to compare substrate specificity both Müller and ganglion cells to various substances such as arginine, ascorbate, carnitine, glutamate, glutamine, and taurine. Among those substances, incubation with [^{3}H]-taurine showed a significant increased in taurine uptake by taurine transporter compared to the other substances ($P < 0.05$). Our data confirm the previous finding of Bridges *et al*., 2001. They showed a high taurine transport in different intact rat retinal cells and rat retinal pigment epithelial cells (9).

To determine the possibility of ion dependency of transporter to taurine in both rat retinal Müller and ganglion cells, uptake of [^{3}H]-taurine was measured in the presence of NaCl, in the absence of Na^+ but in the presence of Cl^- (N-methyl D-glucose chloride) or in the presence of Na^+ but in the absence of Cl^- (sodium gluconate). Our results showed that taurine transport significantly increases in the presence of NaCl. Neither Na^+ nor Cl^- alone was able to increase taurine transport in both types of cells. ß-alanine was also able to inhibit taurine transport competitively in both types of cells. Our finding agrees with the previous finding of Bridges *et al*., 2001 where they reported an ion dependence of the transport process mediated by taurine transporter in rat retinal epithelial cells (9). Miller and Steinberg (1979) reported that taurine uptake was inhibited by ouabain, a Na^+-K^+ ATpase inhibitor (28). Thus, it was suggested that the sodium co-transport of taurine is modulated indirectly through the sodium pump located in the apical membrane of cell.

To determine effect of osmotic stress on taurine transport in rat retinal Müller and ganglion cells, cells were incubated with [^{3}H]-taurine in the presence of graded concentrations of glucose and taurine uptake by RMC1 or RGC5 was measured. Incubation of rat retinal Müller and ganglion cells with [^{3}H]-taurine in a media containing high concentration of glucose showed a significant increase in taurine transport compared with that of low glucose concentration. On the other hand, addition of mannitol to the media containing normal glucose, increase [^{3}H]-taurine uptake to a closed degree to that of the high glucose in both types of cells. These data provide evidence that the increased taurine uptake in the presence of high glucose concentration in both types of cells may be due to osmotic effect but not due to hyperglycemic condition.

Previous studies have shown that concentration of taurine was significantly reduced in retina and retinal pigment epithelium of streptozotocin-induced diabetic rats (29). Our results are in agreement with the previous published data in which the free amino acid levels were also reduced in the retina of diabetic rats (29). The increase in taurine uptake observed in diabetic retina and RPE could be explained by the increase in the transport system rate due to low internal concentration of the amino acid. On the other hand, the increase in taurine uptake may be related to osmotic compensation of volume as taurine and other amino acids has been reported to play an important osmoregulatory role in various tissue including the retina (30)

To address the effect of NO donor on taurine transport, uptake of radiolabel led taurine was assessed in the absence or presence of Nitric oxide donors, SIN-1 and SNAP in both rat retinal Muller cells and ganglion cells. Incubation of rat retinal Müller and ganglion cells with low concentration of SIN-1 or SNAP caused a marked increase in taurine uptake by the transporter. SIN-1 was more potent than SNAP in triggering the transport effect. On the other hand, high concentration of SIN-1 or SNAP tended to kill both rat retinal Müller and ganglion cells and hence decreased the taurine transport. These results agree with the previous finding of Bridges *et al*., 2001 where they reported increase in taurine uptake when ARPE-19 cells incubated with SIN-1 (9). Moreover, treatment of ARPE-9 cells with NO scavengers inhibited SIN-1 stimulated taurine transporter activity suggesting that that the increased taurine transport in retinal cells may be mediated, at least in a part, by the increased NO release by NO donors.

NO is a mediator of many physiological processes, such as vasodilatation and neurotransmission; it is recognized also for its toxic effects (31). NO has been implicated in the pathogenesis of diabetic retinopathy (31). We have shown recently that, in cultured RPE cells, NO stimulates the transport of cystine, the disulfide form of cysteine, which is required for synthesis of the antioxidant glutathione (32). Because NO is known to function as a vasodilator and because diabetic retinopathy has an ischemic component in which NO levels may be decreased, it may seem paradoxical to implicate NO in the pathogenesis of diabetic retinopathy. This paradox is resolved when it is recognized that the increased level of NO observed in the vitreous of diabetic patients is not an early event (25). During the early stages of diabetic retinopathy, typically called the nonproliferative stage, platelets clump together to form small stable aggregates that can lead to capillary closure as a result of a reduction of vascular NO. The nonproliferative stage is followed, however, by a proliferative stage in which new blood vessels are formed. The proliferative stage is associated with increased levels of NO (33). The proliferative stage is also associated with an increase in vascular endothelial growth factor, which has been shown to up regulate NO (34). In addition, proliferative diabetic retinopathy shows elevated intravitreal levels of cytokine tumor necrosis factor, interferon, and interleukin-1 (35). These compounds have been shown also to induce production of NO by the expression of iNOS, which is found in Müller and RPE cells (33, 36).

To address the effect of excitotoxic glutamate on taurine transport in both retinal Müller and ganglion cells,. Incubation of ganglion cells with radioactive taurine in the absence or in presence of graded concentrations of glutamate showed a dose dependent increase in taurine uptake. However, incubation of Müller cells with radioactive taurine in the absence or in presence of graded doses of glutamate showed a non-significant change in taurine uptake at lower concentration while a slight increase has been noticed with the higher concentration. The increase in taurine uptake by ganglion cells is probably to counter act the excitotoxic effects of glutamate. On the other hand, the uptake of taurine by Müller cells did not significantly change probably due to ability of Müller cells to self detoxify glutamate.

Glutamate, a major neurotransmitter, is shown to accumulate in the retina of diabetic animals (37). The metabolism of glutamate is altered in the retina within a few months after the onset of diabetes in rats and first year in human. This alteration remains sub clinical until degeneration proceeds to the point where irreparable vision impairment is incurred. Glutamate accumulation has been shown to increase oxidative stress by increasing oxidation of protein thiol, which in turn can impair glutamate transport system and antioxidant can correct these abnormalities. The mechanism of neurotoxic effect of excessive glutamate is not fully understood and it may be NMDA receptor mediated neurotoxicity. Moreover, it was shown that direct intravitreal injection of glutamate cause 2-3 folds increase in neurodegeneration of ganglion cells where effect can de attenuated using NMDA receptor blocker (38).Müller cells are the principal cells of neuronal retina that span the entire thickness of the retina. Müller cells are implicated in the maintenance of blood retinal barrier at the endothelial lining of retinal micro vessels and play a central role in homeostatic regulation of the retina (39). Müller cells also play an important role in extracellular ion homeostasis and glutamate recycling. Müller cells are key mediator of nerve cell protection, especially via the release of fibroblast growth factor, degradation of excitotoxic glutamate and secretion of antioxidant glutathione (40). Müller cell is the only cell in the retina endowed with glutamine synthetase enzyme so it can transform glutamate taken up via high affinity carriers into glutamine using toxic ammonia and ATP (41). Glutamine is then returned to the neural cells for glutamate re-synthesis. Thus, the released glutamine may contribute to the replenishment of neurotransmitter glutamate in photoreceptor neurons (42).

Müller cells have characteristics that make them both potential targets of diabetes and potential contributors of retinopathy. Müller cells are the primary site of glucose uptake and phosphorylation in the retina. They are endowed with GLUT-1, which permits the transport of glucose. They metabolize glucose intensely through glycolysis to produce lactates that fuel neuronal metabolism (43). Müller cell showed pathological abnormalities in early diabetes such as increase expression of anti-apoptotic molecule Bcl-2 and of the intermediate filament protein Glial Fibrillary Acidic Protein

(GFAP), together with reduced expression of the enzyme glutamine synthetase (44). Müller cell can regulate extracellular concentration of glutamate by its transport through glutamate transporter especially the L-glutamate-L-aspartate transporter and by the properties of glutamine synthetase (45). Müller cell can also protect the ganglion cell from glutamate and NO toxicity, particularly via glutamate uptake and subsequent detoxification by glutamine synthetase. Alternation of the function of Müller cells could affect retinal ganglion cells survival. This is due to the fact that RGCs are unsheathed by RMCs process, which could protect the RGCs from the excitotoxic injuries. Recent study has shown that coculture RGCs with RMCs prevented the neurotoxic effect of glutamate on RGCs as Müller cell can reduce glutamate concentration to a non-toxic level within 30 minutes. These results suggested that functional disorder of glutamate uptake of Müller cells might be one of the early neuronal pathological changes in diabetic retinopathy (46,47).

In summary, our data provide evidence to the importance of retinal taurine in hyperglycemic condition and support the hypothesis of external taurine supplementation for diabetic patients as a prophylactic against diabetic retinopathy and delayed retinal diabetic.

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