Inhibition of Taurine Transport by High Concentration of Glucose in Cultured Rat Cardiomyocytes

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Cultured rat cardiomyocytes were treated with 10, 20, and 30 mmol/L glucose and 30 mmol/L glucose plus protein kinase C (PKC) inhibitor, Chelerythrine. In the 20 and 30 mmol/L glucose-treated cells, taurine contents reduced by 15% and 27% ($P < .05$), respectively, taurine transporter (TAUT) mRNA levels reduced by 47% and 64% ($P < .05$), respectively, and cysteine sulfinate decarboxylase (CSD) mRNA reduced slightly, but not significantly. Time-dependent taurine uptakes reduced in the 10, 20, and 30 mmol/L glucose-treated cells, and time-dependent taurine release reduced in the 30 mmol/L glucose-treated cells. The V_{max} of taurine transport decreased by 18%, 30%, and 35% ($P < .05$) in the 10, 20, and 30 mmol/L glucose-treated cells, respectively, while K_m of taurine transport remained unchanged. When PKC inhibitor, Chelerythrine, combined with 30 mmol/L glucose was treated with the cells, the lowered taurine content, taurine uptake, taurine release, and V_{max} of taurine transport caused by 30 mmol/L glucose were eliminated. These results demonstrate that high glucose considerably and specifically decreases intracellular taurine content, taurine transport activity, and TAUT mRNA, possibly through PKC-mediated transcriptional and posttranslational pathways.

TAURINE (2-aminoethane sulfonic acid) is the most abundant free amino acid in excitable tissues. Intracellular taurine depletion induced by hyperglycemia has been demonstrated in tissues vulnerable to diabetic impairment, such as nerve, lens and mesangial cells.[1,2] Myocardial taurine is related to functioning of the heart. Intracellular taurine depletion in the diabetic heart may result in chronic dysfunction of the heart. Physiologically, high intracellular taurine is maintained by the combination of membrane taurine transporter (TAUT) activity and endogenous synthesis. Synthesis of taurine is, however, low in humans, and in most tissues, intracellular taurine is actively concentrated from the extracellular space by the unidirectional and Na^+-dependent TAUT.[3,4] Cysteine dioxygenase and cysteine sulfinate decarboxylase (CSD) are involved in the biosynthesis pathways, and CSD is thought to be the rate-limiting step in taurine biosynthesis.[5,6] The effect of hyperglycemia on taurine transport and TAUT gene expression is unknown. To elucidate the possible dysfunction of taurine transport in the diabetic heart, we studied the effect of high glucose concentration on taurine transport and TAUT gene expression in cultured rat cardiomyocytes.

MATERIALS AND METHODS

Materials

^{3}H-taurine (925 GBq/mmol) was purchased from NEN (Boston, MA). Mastoparan and taurine were from Sigma (St Louis, MO). Sequences of polymerase chain reaction (PCR) primers are: Tat-S 5′-CAACTTCACTTCGCCTGTGA-3′ and Tat-A 5′-CTTGCTCTTGTGCCATGAAG-3′ used for the quantitation of TAUT mRNA; CSD-S 5′-TGATCCCTGAGGATCTGGAG-3′ and CSD-A 5′-ACTCAAATCCTTCCCGCTTT-3′ used for the quantitation of CSD mRNA; β-actin-S 5′-ATCTGGCACCACACCTTC-3′ and β-actin-A 5′-AGCCAGGTCCAGACGCA-3′ used for sample loading calibration.

Primary Cardiomyocyte Culture and Treatment

Wistar rats were supplied by the Experimental Animal Center at Peking University Healthy Science Center (Clean grade, Certificate No 99-001). Primary culture of ventricular cardiomyocytes was prepared from neonatal rat as previously described,[7] with some modifications. Briefly, the ventricle was removed and digested with 0.025% trypsin-EDTA in phosphate-buffered saline (PBS). The isolated cells were washed with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and preplated twice for 2 hours each. During the preplating procedures, nonmyocytes (mainly fibroblasts) attached readily to the bottom of the culture dish. This was followed by centrifugation at 500 × *g* for 5 minutes. The centrifuged cells were then resuspended in DMEM supplemented with 10% FBS and plated on culture plates at a density of 1.0×10^6 cells/mL in DMEM with 10% bovine calf serum and 10 μmol/L cytosine arabinoside for the first 24 hours. Cells were then cultured in a serum-free medium with 100 μmol/L bromodeoxyuridine for 24 hours. Using this method, we usually obtained myocyte-rich culture with > 90% myocytes as assessed by microscopic observation.

The cells were incubated on 6-well plates in DMEM containing 10% FBS, grew to near confluent, and then starved in serum-free DMEM for 24 hours. The medium was removed and replaced with standard incubation buffer (mmol/L: KCl 3, NaCl 125, KH_2PO_4 12, $MgSO_4$ 1.2, $CaCl_2$ 1.3, $NaHCO_3$ 5, HEPES 20, pH 7.4, and 0.2% bovine serum albumin [BSA]). The following reagents were added: standard incubation buffer (control group); 10, 20, and 30 mmol/L glucose (glucose groups); 5 μmol/L protein kinase C (PKC) inhibitor, Chelerythrine (Chelerythrine group); 30 mmol/L glucose plus 5 μmol/L Chelerythrine (glu_{30} + Chelerythrine group); and 30 mmol/L mannitol (mannitol group). The cells were incubated in 95% air and 5% CO_2 at 37°C for 4 hours, then used for the following experiments.

Cell Viability Assay

After the incubation period, the incubation buffer was removed, replaced with 2.5% trypan blue in PBS, and incubated for 10 minutes at 37°C.[8] The number of dead cells was then determined at × 200

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Submitted August 5, 2002; accepted January 31, 2003.

Supported by The Major Basic Research Program of China (G 200056905).

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0026-0495/03/5207-0050$30.00/0

doi:10.1016/S0026-0495(03)00067-2

magnification by counting those cells that failed to exclude the dye in 3 representative fields for each well. The experiment was performed in triplicate and was repeated a minimum of 2 times for each group.

Measurement of Cell Taurine Content

Taurine was measured by reversed-phase high-performance liquid chromatography (HPLC) after precolumn derivatization with *o*-phthalaldehyde.[9] Briefly, the treated cells were sonicated in 1 mL 6% trichloroacetic acid and centrifuged at 4,000 × *g* for 10 minutes. The supernatant was purified in a dual-bed ion-exchange column (2.5 cm of AG 1-X8 100 to 200 mesh in the chloride form over 2.5 cm of AG 50W-X8 200/400 mesh in the hydrogen form), eluted with 2 mL water, and then lyophilized. Sample or standard was dissolved in 100 μL water before HPLC analysis on a Waters system (Waters, Milford, MA) equipped with a 3.9 × 150 mm Nova-Pak C_{18} column and a model 470 scanning fluorescence detector. Isocratic elution was performed at a flow rate of 2 mL/min using 43% solvent A (0.05 mol/L NaH_2PO_4, pH 5.3, plus 5 mol/L NaOH) mixed with 57% solvent B (0.05 mol/L NaH_2PO_4 in 75% methanol-water). Glutamine, added after ion exchange chromatography, was used as the internal standard. Standard curve of taurine content in the sample concentration range was linear, and recovery of taurine was > 90%. The value of taurine was expressed as nanomoles per milligram protein. Cell protein was measured with the Bradford method using bovine serum albumin as the standard.[10]

Measurement of Taurine Uptake in Cultured Cells

For taurine uptake assay, culture medium was removed and the treated cells were rinsed 3 times with 2-mL aliquots of taurine uptake buffer (mmol/L: HEPES 20, NaCl 140, KCl 5.4, $CaCl_2$ 1.0, $MgSO_4$ 0.8, and glucose 5) at 37°C and equilibrated in 2 mL taurine uptake buffer at 37°C.[11,12] Uptake was initiated by adding uptake buffer containing ^{3}H taurine (100 μmol/L and 18.5 kBq/well). ^{3}H-taurine uptake was performed at 37°C with gentle shaking for 1, 5, 10, and 20 minutes in an atmosphere of 95% O_2 and 5% CO_2. The radioactive buffer was aspirated, cells were washed with ice-cold turine uptake buffer, extracted at room temperature in 1 mL 0.1 mol/L NaOH, sonicated, and measured by liquid scintillation counter (Beckman LS 3800). Nonspecific uptake was measured by adding 10 mmol/L unlabeled taurine into uptake buffer containing 100 μmol/L ^{3}H-taurine. Specific uptake value was obtained by subtracting the nonspecific uptake value from it. Taurine uptake was expressed as picomoles per milligram protein per minute.

The experiment condition of taurine concentration-dependent uptake assay was the same as that of taurine time uptake assay, except taurine uptake buffer contained 0.5 to 100 μmol/L ^{3}H-taurine and the incubation lasted for 5 minutes.

Taurine Release Assay

The treated cells were washed twice with incubation buffer at 37°C and incubated in 2 mL incubation buffer containing 74 kBq/well ^{3}H taurine together with 0.1 mmol/L unlabeled taurine for 10 minutes.[13,14] Subsequently, cells were washed twice with 2 mL incubation buffer at 37°C to remove free ^{3}H-taurine. A total of 2 mL sodium-free incubation buffer (Tris-HCl 20 mmol/L, pH 7.4) was added. An aliquote of incubation buffer (0.25 mL) was removed after 1, 5, 10, and 20 minutes. Radioactivity in the buffer was counted. After that, the cells were taken for ^{3}H radioactive assay. Taurine release assay was expressed as released taurine/total uptake taurine × 100%.

Determination of PKC Activity

The treated cells were harvested by scraping into 0.5 mL ice-cold extraction buffer (50 mmol/L HEPES pH 7.4, 150 mmol/L NaCl, 20 mmol/L EGTA, 1.0 mmol/L dithiothreitol, 0.2 mmol/L phenylmethylsufonyl fluoride, 10% ethylene glycerol, 10 μg/mL leupeptin, 10 μg/mL pepstatin A, 5 μg/mL aprotinin A, 10 nmol/L caliculyn A, and 1% Triton X-100), rotated for 15 minutes at 4°C, and centrifuged at 100,000 × *g* for 1 hour. The supernatant was used for the assay. PKC activity assay was performed according to the method described by Hashimoto and Soderling,[15] with some modifications. Briefly, a 100-μL reaction mixture (50 mmol/L HEPES pH 7.5, 10 mmol/L $MgCl_2$, 1.0 mmol/L $CaCl_2$, 3 μmol/L mastoparan, 35 μg/mL phosphatidylserine, 14 μg/mL 1, 2-diolein, 30 μmol/L PKI-tide, 20 μmol/L syntide-2, 37 kBq/well γ-^{32}P-adenosine triphosphate [ATP]) was preincubated at 30°C for 2 minutes, the reaction was initiated by adding the supernatant containing 20 μg protein, and the incubation was continued for 10 minutes. For determination of protein phosphorylation, 50 μL of the incubated mixture was spotted onto Whatman P81 paper. The dried paper was rinsed with washing-buffer (30% acetic acid, 1% phosphoric acid) twice and once in 95% ethanol, then counted on a scintillation counter. One unit of PKC activity is the phosphorylation of 1 picomole of syntide-2 per milligram of protein per minute under the defined assay conditions.

Measurement of TAUT and CSD mRNA by Competitive Reverse Transcriptase-PCR

We used quantitative reverse transcriptase (RT)-PCR by adding an internal competitive standard into the PCR system to eliminate the errors from variations of exponential amplification and the plateau effect inherited in PCR and to obtain a relatively accurate and reproducible result.[16] PCR was performed using primers Tat-S and Tat-A and a cDNA template transcribed from rat heart total RNA. This yielded a 500-bp fragment of wild-type TAUT cDNA. The internal competitive standard for the measurement of TAUT cDNA was made by PCR method, which had the same sequence and the same primer annealing sites as the 500-bp fragment, except a fragment of 95 bp at the downstream site of Tat-S primer was deleted.[17]

Total RNA was extracted using Trizol (GIBCO, Rockville, MD). A total of 2 μg myocardial total RNA was reverse-transcribed into single strand cDNA using M-MuLV reverse transcriptase and oligo$(dT)_{15}$ primer. Quantitative PCR was performed in a 0.2 mL tube containing heart cDNA 2 μL, 0.182 fmol/L internal competitive standard 1 μL, 5 μmol/L/each Tat-S and Tat-A mixture 1 μL, 2.5 mmol/L/each dNTP mixture 1 μL, 1.5 mmol/L $MgCl_2$, 10× PCR buffer 2.5 μL, and 1.25 U Taq DNA polymerase, in a total volume of 25 μL. PCR was run at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 40 seconds for 40 cycles. The PCR product was separated in a 1.5% agarose gel and stained by ethidium bromide. The ratio of optical density of the 2 DNA bands was measured under ultraviolet (UV) light. A standard curve of the ratio was drawn using the same conditions as described above, except that heart cDNA was changed to a series dilution of the plasmid containing the 500 bp DNA fragment.[18] The amount of TAUT mRNA in sample was then obtained from the standard curve. Amplification of TAUT cDNA was confirmed by the digestion of the PCR product with restriction enzyme Acc I, which cuts the cDNA into 171 bp and 329 bp. To calibrate the sample loaded in PCR mixture, β-actin cDNA was measured after the quantitative PCR. A total of 2 μL PCR product was reamplified using the 2 rat β-actin primers at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds for 20 cycles, and the optical density of the β-actin band (291 bp) was measured. The relative amount of β-actin cDNA in the loaded sample was obtained from a premade standard curve of β-actin cDNA measurement. The calibrated amount of TAUT mRNA was used for further analysis.

For CSD mRNA determination, a fragment of 552 bp wild-type rat CSD cDNA and an internal competitive standard of 447 bp were obtained using similar procedures as described above. PCR was run at 94°C for 40 seconds, 55°C for 30 seconds, and 72°C for 40 seconds for

Table 1. Taurine Content, PKC Activity, V_{max}, and K_m of ^{3}H-Taurine Transport in Cultured Rat Myocardial Cells

Group	Cellular Taurine (nmol/mg pr)	Cellular PKC (pmol/mg pr/mins)	V_{max} of ^{3}H-Taurine Transport (pmol/mg pr)	K_m of ^{3}H-Taurine Transport (μmol L)
Control	250 ± 26	64.2 ± 4.8	36.7 ± 3.1	5.2 ± 0.6
Chelerythrine	238 ± 25	38.1 ± 4.0‡§	37.0 ± 4.0	5.3 ± 0.5
10 mmol/L glucose	231 ± 22	70.2 ± 5.2	30.1 ± 2.9*	5.5 ± 0.5‡
20 mmol/L glucose	212 ± 20*	88.6 ± 6.8*	25.8 ± 2.8*	6.1 ± 0.5‡
30 mmol/L glucose	182 ± 12*	96.4 ± 8.2*	23.8 ± 2.0*	5.5 ± 0.5‡
Glu_{30} + chelerythrine	230 ± 18†	46.2 ± 5.0§	38.8 ± 3.0†	6.1 ± 0.5§
Mannitol	262 ± 22	58.7 ± 5.4	36.4 ± 3.2	5.3 ± 0.5

NOTE. Data are shown as mean ± SEM.
*$P < .05$ *v* control group.
†$P < .05$ *v* 30 mmol/L glucose group.
‡$P < .01$ *v* control group.
§$P < .01$ *v* 30 mmol/L glucose group.

30 cycles. Amplification of CSD cDNA was comfirmed by digestion with the restriction enzyme BamHI, which cuts the cDNA into 332 bp and 220 bp.

Statistical Analysis

Data were expressed as mean ± SEM from at least 6 independent experiments. Apparent V_{max} and K_m were determined by nonlinear regression fit to Michaelis-Menten equation using the nonlinear regression program (GraphPad Prism software). Differences among experimental groups were detected by analysis of variance, and the differences between groups were assessed by the Student-Newman-Keuls test. Significance was defined below the .05 level.

RESULTS

Cell Viability

Cell viability was unaffected by the treatment of different reagents. Cell viability of the 7 groups was from 97% ± 1.2% to 99% ± 2.0%. No statistical significance was found among the groups ($P > .05$).

Taurine Content

Taurine contents were decreased by 15% and 27% ($P < .05$) in the 20 and 30 mmol/L glucose groups as compared with that in the control group. The decreased taurine content of the 30 mmol/L glucose group was completely eliminated by the addition of PKC inhibitor, Chelerythrine, as shown in taurine change of the Glu_{30} + chelerythrine group. Taurine content was not affected by the treatment of 30 mmol/L mannitol or 3 μmol/L chelerythrine (Table 1).

Time-Dependent Taurine Uptake and Release and TAUT Kinetic Characterization

^{3}H-taurine time uptake and release. Taurine uptakes in the 7 groups increased with incubation time and were saturated after 5 minutes. Taurine uptake was low in 10, 20 ($P < .05$) and 30 ($P < .05$) mmol/L glucose groups. Taurine release was abnormal only in the 30 mmol/L glucose group, in which taurine release was decreased by 27%, 20%, 20%, and 18% at 1, 5, 10, and 20 minutes, respectively, as compared with that of the controls ($P < .05$). Taurine uptake and release were unaffected by the treatment of 30 mmol/L mannitol or PKC inhibitor (Fig 1).

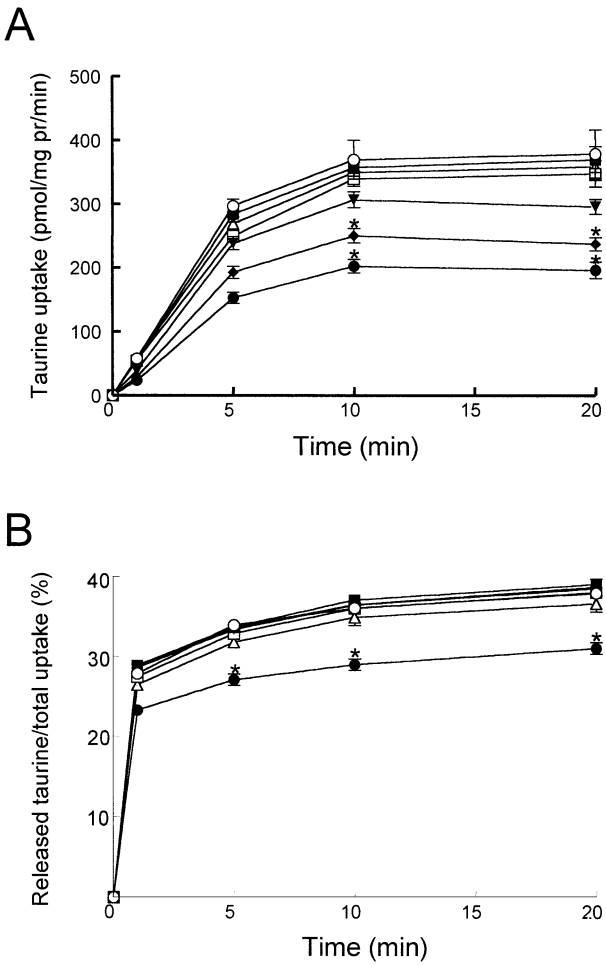

Fig 1. (A) Time-dependent taurine uptake (at taurine 100 μmol/L) and (B) release; n = 6; mean ± SEM. *$P < .05$ *v* control group. Control (○), chelerythrine (■), 10 mmol/L glucose (▼), 20 mmol/L glucose (◆), 30 mmol/L glucose (●), Glu_{30} + chelerythrine (□), and mannitol (△).

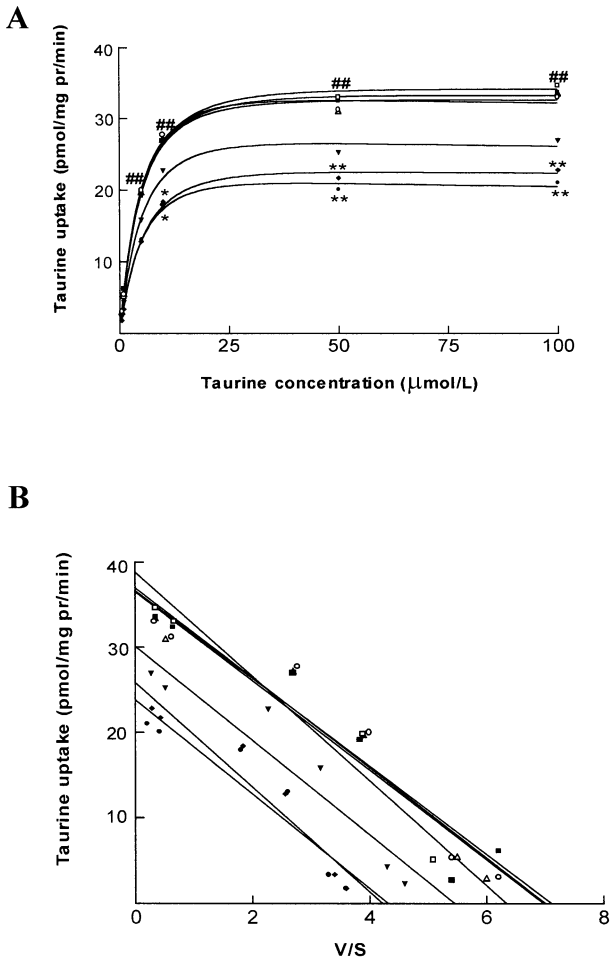

Fig 2. (A) Taurine uptake at 6 substrate concentrations (0.5, 1.0, 5.0, 10, 50, and 100 μmol/L) and (B) Eadie-Hofstee plot of the data; n = 6, mean ±SEM. *$P < .05$ *v* control group. Control (○), chelerythrine (■), 10 mmol/L glucose (▼), 20 mmol/L glucose (◆), 30 mmol/L glucose (●), Glu + chelerythrine (□), and mannitol (△).

Kinetic characterization. The K_m of Na^+-dependent taurine uptake was 5.2 ± 0.6 μmol/L, and V_{max} was 36.7 ± 3.14 pmol · mg pro^{-1} · $minute^{-1}$ in the control group (Table 1 and Fig 2). Exposure of cells to 10, 20, and 30 mmol/L glucose resulted in the decrease of dose-dependent taurine uptakes (Fig 2A). However, there was no change in the 30 mmol/L mannitol group. Kinetic characterization of TAUT in the 7 groups is shown in Fig 2B. Exposure to 10, 20, and 30 mmol/L glucose did not affect the TAUT K_m, but the V_{max} was significantly decreased by 18%, 30%, and 35% ($P < .05$), respectively, as compared with those of the control group (Table 1). These data indicate that high glucose-induced intracellular taurine depletion results, at least partly, from dysfunction of TAUT as manifested by the reduction of TAUT V_{max}.

TAUT and CSD mRNA

In the 20 and 30 mmol/L glucose groups, myocardial TAUT mRNA was significantly reduced by 47% and 64%, respectively, as compared with that of the control group ($P < .05$) (Fig 3). CSD mRNA was decreased, but not significantly ($P > .05$), by 19% and 17%, respectively (Fig 4).

Glucose-Sensitive Signal Transduction Pathway on TAUT Activity

To evaluate the regulation of TAUT by PKC, taurine uptake and release were measured in cardiomyocytes treated with the PKC inhibitor, Chelerythrine. PKC inhibitor, Chelerythrine (5 μmol/L, 4 hours) used in incubation with the 30 mmol/L glucose treatment changed the lowered taurine uptake and release induced by 30 mmol/L glucose to nearly normal ($P < .05$) (Figs 1A and B and 2A). In the 30 mmol/L glucose + chelerythrine group, V_{max} of taurine transport was increased by 63% ($P < .01$) as compared with that of the 30 mmol/L glucose group (Table 1). These data support the hypothesis that TAUT activity is regulated through PKC pathway and activation of PKC inhibits TAUT activity.

DISCUSSION

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin function, or both.

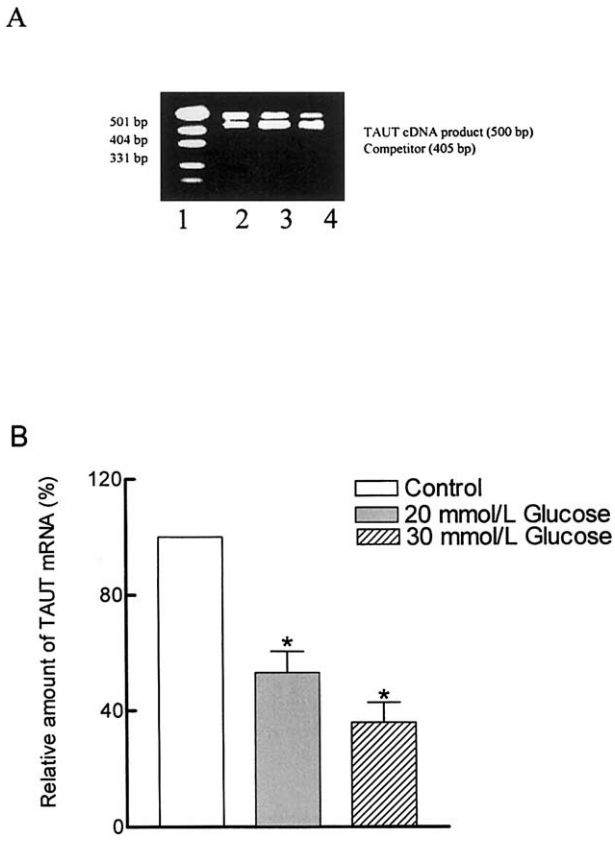

Fig 3. (A) An example of a quantitative RT-PCR experiment. PCR products of TAUT cDNA were separated in a 1.5% agarose gel. Lane 1, standard of DNA molecular weight; lane 2, a control; lane 3, a 20-mmol/L glucose sample; lane 4, a 30-mmol/L glucose sample. (B) Measurement of myocardial TAUT cDNA by quantitative RT-PCR from 4 independent experiments. Mean ± SEM. *$P < .05$ *v* control group.

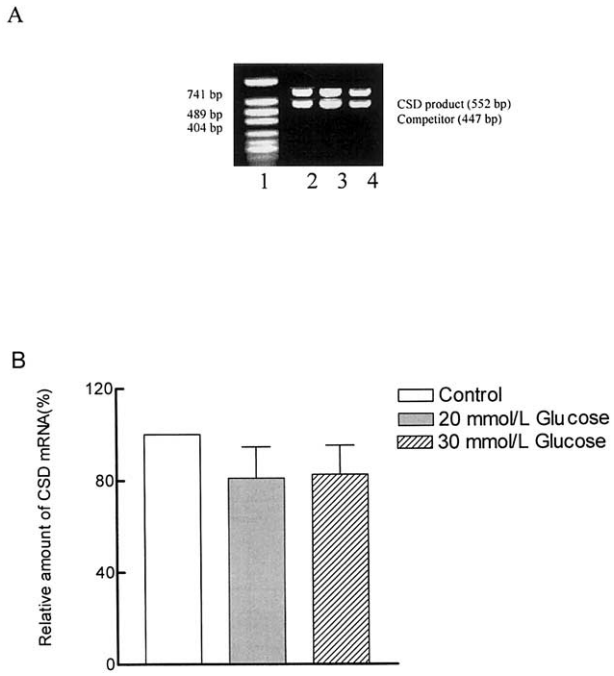

Fig 4. (A) An example of a quantitative RT-PCR experiment. PCR products of CSD cDNA were separated in a 1.5% agarose gel. Lane 1, standand of DNA molecular weight; lane 2, a control; lane 3, a 20-mmol/L glucose sample; lane 4, a 30-mmol/L glucose sample. (B) Measurement of myocardial CSD mRNA by quantitative RT-PCR from 4 independent experiments. Mean ± SEM.

Many of the complications in diabetes mellitus, such as renal failure, cardiomyopathy, vascular damage, and retinal degeneration have been attributed to chronic hyperglycemia.[19] Cardiovascular diseases represent the most common and devastating complications in diabetes mellitus. About 20% of diabetic patients died of myocardial infarction.[20,21] Taurine is the most abundant free amino acid in excitable tissues, such as nerve and muscle.[22,23] Taurine content is highest in the heart (~40 mmol/L). The physiologic role of taurine may therefore be more important in the heart than in other tissues. Taurine is thought to modulate ion movement across sarcolemma, especially Na^+ ion, providing protection against abrupt ion imbalance that may otherwise lead to cell damage. It has also been reported that taurine modulates protein phosphorylation and Ca^{2+} movement in the heart.[24,25] Intracellular taurine is accumulated via 2 mechanisms, transport from extracellular space and biosynthesis within the cell. The high intra- and extracellular taurine gradient is maintained by TAUT.[26] Endogenous taurine is synthesized from its amino acid precursor, methionine and cysteine. CSD is considered as the rate-limiting enzyme in the biosynthesis of taurine. CSD may thus represent a key enzyme in the biological effects induced by taurine.[27] Animals, such as humans, cats, and certain monkeys are unable to synthesize sufficient taurine, and must rely on dietary source to maintain their requirement. The kidney is the most important organ in the metabolic balance of taurine. Taurine transport at the brush border membrane of renal proximal tubules is regulated by circulating taurine and maintains the taurine body pool by renal reabsorption. Many functions of taurine, such as membrane stabilization, detoxification, antioxidation, osmoregulation, calcium modulation, and acting as an inhibitory neurotransmitter or a neuromodulator, have been proposed.[28] In diabetes, taurine may inhibit the pathologic protein phosphorylation and oxidative damage, and intracellular taurine deficiency may result in degeneration of the cells. Transport of taurine across cell membrane is a critical step to play its physiologic roles. For example, the biophysical and biochemical properties of taurine make it an excellent factor for osmoregulation.

Our results demonstrate that high glucose rapidly and specifically causes depletion of intracellular taurine. We further studied the mechanism by which high glucose interferes with intracellular taurine accumulation in cultured rat cardiomyocytes. In the cultured cells, high glucose lowered the intracellular taurine content, which may be secondary to the high glucose-induced impairment of taurine uptake and release. Changes of TAUT mRNA were parallel to those of ^{3}H-taurine release and uptake. High glucose decreased the V_{max} of taurine transport. High glucose may also cause hyperosmolarity. Therefore, we used 30 mmol/L mannitol, a metabolically dull carbohydrate, to treat cultured cells to evaluate the potential effect of hyperosmolarity on intracellular taurine content and TAUT activity. Results of the 30 mmol/L mannitol group indicate that osmolarity change itself has no effect on intracellular taurine content and TAUT activity (Fig 1 and 2). Taurine also functions as an osmoregulator. Extracellular hyerosmolarity, such as 30 mmol/L mannitol, should induce the increase of intracellular taurine. However, mannitol in this study had no effect on taurine content and TAUT and PKC activities. As a contract organ, taurine transport in the heart may be relatively insensitive to extracelluar osmolarity. In this aspect, the function of heart TAUT may be different from that of TAUT in the brain and kidney. These results suggest that high glucose causes the downregulation of the TAUT gene and the decrease of TAUT activity, thus the insufficiency of intracellular taurine. In other words, high glucose may disrupt taurine transport across the cardiac cell membrane. Our results from cell culture model may be helpful for further understanding of diabetic heart diseases.

Kinetic characterization of TAUT in this study confirms the existence of a high-affinity taurine transport system in cultured rat cardiomyocytes as reported by others in intact baboon retinal pigment epithelial cells and cultured human retinal pigment epithelial cells.[9,29] In this study, high glucose treatment caused the lowering of V_{max} of taurine transport without change of K_m, indicating the change of quantity rather than quality of TAUT on cell membrane. Theoretically, there may be 2 pathways through which high glucose affects TAUT activity: transcriptional and posttranslational. Many factors may be involved in the 2 pathways. In this study, we used the PKC inhibitor, Chelerythrine, to further study the mechanism by which high glucose influences taurine metabolism in cardiac cells. We found that the PKC inhibitor completely eliminated the changes of taurine uptake and release and the depletion of intracellular taurine induced by the 30 mmol/L glucose treatment. PKC inhibitor also reversed the change of V_{max} of taurine transport. Consequently, PKC may play a key role in the high glucose-induced derangement of taurine metabolism.

Presumably, high glucose stimulates the PKC activity and inhibits the transcription of TAUT through PKC phosphorylation of its transcription factors. PKC may also disturb taurine metabolism through phosphorylation of other proteins, such as signal transduction proteins or even TAUT itself. More experiments are needed to understand PKC in the pathogenesis of high glucose-induced impairment.

TAUT belongs to the gene family of Na^+- and/or Cl^--dependent neurotransmitter transporters. Rat TAUT mRNA encodes a protein of 621 amino acids exhibiting 12 putative transmembrane segments and multiple potential PKC and protein kinase A (PKA) phosphorylation sites. Changes in signal transduction pathways potentially affect the overall effect of TAUT activity. TAUT activity is regulated by PKC, PKA, and Ca^{2+}/calmodulin-dependent protein kinases (CaMK). PKC activation has been reported to inhibit TAUT activity.[30] Our results also confirm this observation. A critical site for phosphorylation by PKC was found in TAUT located at serine-322 in the fourth intracellular domain.[31] Phosphorylation of this site inhibits TAUT activity. The role of PKA in mediating the effect is unclear, as both PKA activation and inhibition decrease TAUT activity. The paradoxical effect of PKA on TAUT activity may reflect multiple and functionally variable PKA-mediated phosphorylation sites on TAUT protein.

CSD belongs to a family of pyridoxal 5′-phosphate (PLP)-dependent enzymes and is a rate-limiting enzyme in the biosynthesis of taurine. CSD mRNA encodes a 55.2-kd protein of 493 amino acids.[32] Our results shows that high glucose may also induce, but to a lesser degree, the downregulation of CSD gene expression. However, it seems unlikely that the decrease of intracellular taurine synthesis significantly contributes to the depletion of intracellular taurine.

In conclusion, our study demonstrates that high glucose significantly and specifically decreases intracellular taurine content, TAUT activity, and mRNA, possibly through PKC-mediated transcriptional and posttranslational pathways. Taurine is an ample substance in the body, and it should have no toxicity, drug dependence, or tolerance with a very wide range of safety when used clinically. Therefore, further studies should be performed on its clinical applications as a reagent. These findings of the dysfunction of taurine transport may contribute to understanding the mechanism of the diabetic heart impairment and finding new strategies for the prevention and therapy of heart complications in diabetes.

REFERENCES

1. Stevens MJ, Lattimer SA, Kamijo M, et al: Osmotically-induced nerve taurine depletion and the compatible osmolyte hypothesis in experimental diabetic neuropathy in the rat. Diabetologia 36:608-614, 1993

2. Trachtman H, Futterweit S, Bienkowski RS: Taurine prevents glucose-induced lipid peroxidation and increased collagen production in cultured rat mesangial cells. Biochem Biophys Res Commun 191: 759-765, 1993

3. Bitoun M, Levillain O, Tappaz M: Gene expression of the taurine transporter and taurine biosynthetic enzymes in rat kidney after antidiuresis and salt loading. Pflugers Arch Eur J Physiol 442:87-95, 2001

4. Xiaobin H, Andrea MB, Russell WC: Adaptive regulation of MDCK cell taurine transporter (pNCT) mRNA: Transcription of pNCT gene is reguiated by external taurine concentration. Biochim Biophys Acta 1351:296-304, 1997

5. Reymond I, Bitoun M, Levillain O, et al: Regional expression and histological localization of cysteine sulfinate decarboxylase mRNA in the rat kidney. Histochem Cytochem 48:1461-1468, 2000

6. Beetsch JW, Olson JE: Taurine synthesis and cysteine metabolism in cultured rat astrocytes: Effects of hyperosmotic exposure. Am J Physiol 274:C866-C874, 1998

7. Takahashi N, Seko Y, Noiri E, et al: Vascular endothelial growth factor induces activation and subcellular translocation of focal adhesion kinase (p125FAK) in cultured rat cardiac myocytes. Circ Res 84:1194-1202, 1999

8. Motterlini R, Foresti R, Intaglietta M, et al: NO-mediated activation of heme oxygenase: Endogenous cytoprotection against oxidative stress to endothelium. Am J Physiol 270:H107-114, 1996

9. Jones BN, Paabo S, Stein S: Amino acid analysis and enzymatic sequence determination of peptide by an improved *o*- phthaldialdehyde precolumn labeling procedure. J Liq Chromatogr 4:565-586, 1981

10. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantitative of protein unitilizing the principle of protein dye binding. Anal Biochem 72:123-125, 1976

11. Chang RC, Stadlin A, Tsang D: Effects of tumor necrosis factor alpha on taurine uptake in cultured rat astrocytes. Neurochem Int 38:249-254, 2001

12. Stevens MJ, Hosaka Y, Masterson JA, et al: Downregulation of the human taurine transporter by glucose in cultured retinal pigment epithelial cells. Am J Physiol 277:E760-771, 1999

13. Benrabh H, Bourre JM, Lefauconnier JM: Taurine transport at the blood-brain barrier: An in vivo brain perfusion study. Brain Res 692:57-65, 1995

14. Zielinska M, Hillgier W, Law RO, et al: Effects of ammonia in vitro on endogenous taurine efflux and cell volume in rat cerebrocortical minislices: Influence of inhibitors of volume-sensitive amino acid transport. Neuroscience 91:631-638, 1999

15. Hashimoto Y, Soderling TR: Calcium, calmodulin-dependent protein kinase II and calcium phospholipid-dependent protein kinase activities in rat tissues assayed with a synthetic peptide. Arch Biochem Biophys 252:418-425, 1987

16. Francesco SC, Michael EZ, Alan RS: A rapid and versatile method to synthesine internal standards for competitive PCR. Nucleic Acids Res 21:1047, 1993

17. Paul DS, James WL: Competitive PCR. Nature 359:557-558, 1992

18. Paul DS, James WL: Standard Curve Quantitative Competitive RT-PCR (SC-QC-RT-PCR): A Simple Method to Quantify Absolute Concentration of mRNA From Limited Amounts of Sample. Natick, MA, Biotechniques Books, 1998, pp 91-101

19. Militante JD, Lombardini JB, Schaffer SW: The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. Cardiovasc Res 46:393-402, 2000

20. Kannel WB, McGee DL: Diabetes and cardiovascular disease. The Framingham study. J Am Med Assoc 241:2035-2038, 1979

21. Mahgoub MA, Abd-Elfattah AS: Diabetes mellitus and cardic function. Mol Cell Biochem 180:59-64, 1998

22. Macaione S, Ruggeri P, Tucci G: Free amino acids in developing rat retine. J Neurochem 22:887-891, 1974

23. Orr HT, Cohen AJ, Lowry OH: The distribution of taurine in the vertebrate retina. J Neurochem 26:609-611, 1976

24. Lombardini JB: Quantitative analysis of the combination dose-effects of taurine and taurine analogues on the phosphorylation of an approximately 44-Kd protein present in a mitochondrial subfraction of rat heart. J Cardiovasc Pharmacol 28:107-114, 1996

25. Punna S, Ballard C, Hamaguchi T, et al: Effect of taurine and

methionine on sarcoplasmic reticular Ca^{2+} transport and phospholipid methyltransferase activity. J Cardiovasc Pharmaacol 24:286-292, 1994

26. Sammanda R, Frederick HL, Virendra BM, et al: Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta. Biochem J 300:893-900, 1994

27. Bitoun M, Tappaz M: Gene expression of the transporters and biosynthetic enzymes of the osmolytes in astrocyte primary cultures exposed to hyperosmotic conditions. GLIA 32:165-176, 2000

28. Huxtable RJ: Physiological action of taurine. Physiol Rev 172: 101-146, 1992

29. Salceda R: Insulin-stimulated taurine uptake in rat retina and retinal pigment epithelium. Neurochem Int 35:301-306, 1999

30. Takeuchi K, Toyohara H, Sakaguchi MA: Hyperosmotic stress-induced mRNA of carp cell encodes Na^{+} and Cl^{-}-dependent high affinity taurine transporter. Biochim Biophys Acta 1464:219-230, 2000

31. Han X, Budreau AM, Chesney RW: Ser-322 is a critical site for PKC regulation of the MDCK cell taurine transporter (pNCT). J Am Soc Nephrol 10:1874-1879, 1999

32. Tappaz M, Bitoun M, Reymond I, et al: Characterization of the cDNA coding for rat brain cysteine sulfinate decarboxylase: Brain and liver enzymes are identical proteins encoded by two distinct mRNAs. J Neurochem 73:903-912, 1999