ISSN 1671-4083
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Dysfunction of myocardial taurine transport and effect of taurine supplement in rats with isoproterenol-induced myocardial injury[1]

SHI Yan-Rong[2,3], BU Ding-Fang[2], QI Yong-Fen[2,3], GAO Lin[2,3], JIANG Hong-Feng[2,3], PANG Yong-Zheng[2], TANG Chao-Shu[2,3,4], DU Jun-Bao[5]

[2]Institute of Cardiovascular Disease Research, Peking University First Hospital, Beijing 100034; [3]Institute of Cardiovascular Research, Peking University, Beijing 100083; [5]Department of Pediatrics, Peking University First Hospital , Beijing 100034, China

KEY WORDS isoproterenol; myocardium; taurine; transportation; cysteine proteinases; messenger RNA

ABSTRACT

AIM: To study the alterations of myocardial taurine transport function, taurine transporter (TAUT), and cysteine sulfinate decarboxylase (CSD) mRNA as well as effect of exogenous taurine in rats with isoproterenol (ISO)-induced cardiomegaly. **METHODS:** [^{3}H]-Taurine uptake and release on myocardium were determined. Binding sites of [^{3}H]-taurine for myocardial sarcolemma were measured. TAUT and CSD mRNA levels were assayed using competitive quantitative reverse transcription polymerase chain reaction (RT-PCR). **RESULTS:** ISO group as compared with control group, myocardial taurine uptake markedly reduced, taurine release obviously increased; B_{max} value of [^{3}H]-taurine binding on cardiac sarcolemma reduced by 42 % (P<0.05); TAUT and CSD mRNA levels decreased by 40 % and 38 % (P<0.05), respectively. ISO+taurine group as compared with ISO-treated group, the amounts of taurine uptake and TAUT mRNA returned to normal; taurine release reduced; B_{max} increased by 92 % (P<0.01), and CSD mRNA content augmented by 23 % (P<0.05). There were no statistical differences of K_d values among the four groups (P>0.05). **CONCLUSION:** The data indicate that the failure to generate sufficient TAUT on myocardial sarcolemma may be the fundamental abnormality in ISO-induced cardiac injury. The mechanism by which administration of taurine considerably improves ISO-induced cardiac damage is probably to increase the expression of TAUT gene and recover taurine transport function.

INTRODUCTION

Taurine (2-aminoethane sulfonic acid) is a sulfur-containing amino acid that is the most abundant free amino acid in excitable tissues and cells. A 70-kg human contains up to 70 g of taurine. There is a high gradient of taurine concentration between intra- and extracellular space[1]. For example, the gradient is 200:1 in rat heart. Cysteine dioxygenase (CDO) and cysteine sulfinate decarboxylase (CSD) are considered involving biosynthetic pathway that leads to taurine from cysteine and methionine, where CSD is thought to be the rate-limiting enzyme in taurine biosynthesis[2]. In humans, cats, and certain monkeys, taurine biosynthesis is very low and must rely on dietary sources to maintain their body loads. This gradient is maintained by

[1] Project supported by the State Major Basic Research Program of China, No G 200056905.
[4] Correspondence to TANG Chao-Shu.
Phn 86-10-6617-6255 or 86-10-6209-2183.
Fax 86-10-6617-6255. E-mail tangchaoshu@263.net.cn
Received 2002-02-08 Accepted 2002-07-11

taurine transporter (TAUT) through the transportation of taurine, ie, the taurine transportation on cellular plasma membrane. TAUT is usually considered as one of the key processes to maintain higher intracellular taurine concentration and to play its physiological roles. Taurine has extensively biological functions, such as stabilizing cell membrane, scavenging oxygen free radicals, regulating intracellular osmostasis, and maintaining intracellular Ca^{2+} concentration. Exogenous administration of taurine possesses a preventive and therapeutic effect on a variety of cardiovascular diseases, such as myocardial ischemia, ischemia-reperfusion injury, hypertension, cardiac hypertrophy, and congestive heart failure, but the mechanism remains to be unclear. Transport of taurine across cell membranes is one of the major processes to play its physiological roles. It seems unlikely that taurine used for the treatment is simply a replenishment therapy, because taurine is abundant in the body, and no taurine obvious deficiency has been found in these cardiovascular diseases. These facts lead us to postulate that there may be a dysfunction of taurine transport in heart diseases, which causes the impairment of myocardial cells. To understand the possible mechanism responsible for the taurine transport dysfunction, we studied the alterations of myocardial taurine transport, and taurine transporter (TAUT) and CSD mRNA contents as well as effect of exogenous taurine in rats with isoproterenol (ISO)-induced cardiomegaly.

MATERIALS AND METHODS

Materials [^{3}H]-Taurine (925 GBq/mmol) was purchased from DuPont New England Nuclear, and isoproterenol and taurine were purchased from Sigma Co (St Louis, MO, USA). Sequences of oligonucleotide primers are: Tat-S 5'-CAACTTCACTTCGCCTG TGA-3' and Tat-A 5'-CTTGCTCTTGTGCCATGAAG-3' used for the quantitation of taurine transporter mRNA, and Tat-T 5'-TCACTTCGCCTGTGACGCTCTGCCTCCT-CTTAG-TC-3' used for making its internal competitive standard; CSD-S 5'-TGATCC CTGAGGATCTGGAG-3' and CSD-A 5'-ACTCAAATCCTTCCCGCTTT-3' used for the quanti-tation of CSD mRNA, and CSD-T 5'-AAATCCTTCCC-GCTT-TTGAGCCA CAGCTTCA-GACAG-3' used for making its internal competitive standard; β-actin-S 5'-ATC-TGGCACCACACCTTC-3' and β-actin-A 5'-AGCCAG-GTCCAGACGCA-3' used for sample loading calibration.

Preparation of animals Forty male Wistar rats (weight 190 g±10 g) were supplied by the Experimental Animal Center at Peking University Health Science Center (Grade II, Certificate № 99-001). Rats were randomly and equally divided into four groups: group 1, control; group 2, taurine treated; group 3, ISO treated; group 4, ISO and taurine co-treated. Rats were fed commercial rat food. Taurine-treated animals (group 2 and 4) received drink of 2 % taurine. ISO was sc administered (5 mg· kg^{-1}· d^{-1}) once a day (group 3 and 4). Rats in group 1 and 2 were given sc injections of 0.9 % saline instead of ISO. These treatments were lasted for 10 d[3].

Pathological examination On d 11, the animal was anesthetized, plasma was separated from the blood immediately, and heart was weighed. The apex of the heart was taken for pathological examination microscopically after hematoxylin-eosin stain.

Measurement of taurine in heart tissue and plasma[3] Myocardium 0.2 g in heart apex was homogenized with normal saline. The homogenate was mixed with 5 volumes of 10 % sulphosalicylic acid, and centrifuged at 1000×*g* for 10 min at 4 ℃. Taurine contents in the tissue supernatant and plasma were determined by a high performance amino acid analyzer (Hitachi Model 835).

Taurine uptake assay[4] Ventricular tissues were cut into 0.3 mm slices and put into 2 mL ice-cold Krebs-Henseleit (K-H) solution containing taurine 200 μmol/L and 18.5 kBq [^{3}H]-taurine. The mixture was shaken and incubated for 1, 2, 5, 10, and 15 min at 37 ℃ during equilibration of 95 % air/5 % CO_2. The tissue slices were rinsed with ice-cold K-H solution for three times and digested with formic acid. The amount of [^{3}H]radioactivity in each fraction was measured using a Beckman LS 3800 liquid scintillation spectrometry. Specific uptake value was obtained by subtracting nonspecific uptake value from it and expressed as nmol/g protein.

Taurine release assay[5] The preparation of ventricular tissue and the condition of [^{3}H]-taurine incubation were the same as in taurine uptake assay, except the incubation lasted for 5 min. After the incubation, the slices were rinsed with ice-cold K-H solution for 3 times to remove free [^{3}H]-taurine, then incubated in 2 mL of sodium-free buffer (Tris-HCl 20 mmol/L, pH 7.4) at 37 ℃ for 1, 2, and 5 min. The supernatant was taken for [^{3}H]radioactive assay. After digestion with formic acid, the incubated tissues were used for

[^{3}H]radioactive assay. Taurine release assay was expressed as: released taurine/total uptake taurine×100 %.

[^{3}H]-Taurine binding assay for myocardial sarcolemma The preparation of crude sarcolemma of myocardium was prepared according to the method of Wolff with some modifications[6]. The activity of Na^+-K^+ ATPase was determined as the maker enzyme of sarcolemma[7]. Myocardial membrane fraction 50 μL (200 μg member protein) was mixed with 100 μL of K-H solution containing different concentration (10-400 μmol/L) of taurine and 18.5 kBq [^{3}H]-taurine. The mixture was shaken and incubated at 37 °C for 5 min, uptake was terminated by the addition of 2 mL of ice-cold K-H solution followed by rapid filtration through a Millipore filter (DAWP type, 0.45 mm pore size). Radioactivity on the filter was determined by a liquid scintillation spectrometry. Specific binding value was obtained by subtracting the nonspecific binding value from it. [^{3}H]-Taurine binding capacity expressed as nmol/g protein, the maximal binding capacity (B_{max}), and the constant K_d were obtained according to the method of Scatchard plot.

Plasma lactate dehydrogenase (LDH) activity was measured on an automatic analyser. Myocardial malondialdehyde (MDA) was determined using thiobarbituric acid test[8].

Measurement of TAUT and CSD mRNA by competitive reverse transcription polymerase chain reaction (RT-PCR)[9,10] PCR was performed using primers Tat-S and Tat-A and a cDNA template transcribed from rat kidney total RNA. This yielded a 500-bp fragment of wild-type TAUT cDNA. The PCR product from the second round PCR was the internal competitive standard that had the same sequence as the 500 bp fragment, except a fragment of 95 bp at the downstream site of Tat-S primer was deleted.

The total RNA was extracted using Trizol (Gibco, Rockville, MD) reagent. Myocardial or vascular total RNA 2 μg 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, 1 μL Tat-S and Tat-A primer mixture 5 μmol/L, 1 μL dNTP mixture 2.5 mmol/L, $MgCl_2$ 1.5 mmol/L, 10×PCR buffer 2.5 μL, and 1.25 unit *Taq* DNA polymerase, in a total volume of 25 μL. After denaturing at 95 °C for 5 min, PCR was run at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s by 40 cycles. A standard curve of the ratio was drawn using the same conditions as described above, except heart cDNA was changed to a series dilutions of the plasmid containing the 500 bp DNA fragment[11]. The amount of TAUT mRNA in sample was then obtained from the standard curve. Amplification of TAUT cDNA was confirmed by digestion of the PCR product with restriction enzyme ACC I, which cuts the cDNA into 171 and 329 bp. To calibrate the sample loaded in PCR mixture, β-actin cDNA was quantitated after the quantitative PCR. PCR product 2 μL was amplified again using the two rat β-actin primers at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s by 20 cycles, and extend at 72 °C for 7 min and the optical density of the β-actin band (291 bp) was measured. Relative amount of β-actin cDNA in loaded sample could be obtained from a pre-made standard curve of β-actin cDNA measurement. Calibrated amount of TAUT cDNA 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 the similar procedures as described above. PCR was run at 94 °C for 40 s, 55 °C for 30 s, and 72 °C for 40 s by 30 cycles. Amplification of CSD cDNA was confirmed by digestion with the restriction enzymes *BamH* I, which cuts its cDNA into 332 bp and 220 bp.

Statistical analysis Data were expressed as mean±SD of at least 6 independent experiments. Apparent K_d and B_{max} values were determined by nonlinear regression fit to the Michaelis-Menten equation using a non-linear regression program (GraphPad Prism software program). A one-way ANOVA method were used to analyze the K_d and B_{max} values from experiments. Multiple comparisons of the significant ANOVA were performed by Tukey multiple comparison test. $P<0.05$ was considered as statistically significant.

RESULTS

ISO-induced myocardial injury During the experimental period, the overall mortality was four rats in ISO group and two rats in ISO plus taurine group. No death occurred in rats treated with taurine alone or in control rats. In ISO-treated group, the heart became enlarged markedly. The ratio of heart weight to body weight (heart coefficient) was significantly increased by 54 % ($P<0.01$), as compared with control group. Un-

der a light microscope observation (40×10) (Fig 1), the lesions of histiocytes were apparent in all hearts taken from ISO-treated rats. Myocardial cells were hypertrophic, and the average dimension of myocardial cells was 6.6 times larger than that of control group [(43±9) μm *vs* (6.6±0.9) μm, $P<0.01$]. Some necrosis cells and a lot of tissue fibrosis were founded. Myocardial MDA formation and plasma LDH activity were increased by 43 % ($P<0.01$) and 138 % ($P<0.01$), respectively, as compared with control group. However, the animals given ISO plus taurine showed a lesser increase in heart coefficient ($P<0.05$ *vs* ISO group), and the myocardial hypertrophy and necrosis cells were trivial. Taurine treatment protected against the increased in myocardial MDA contents stimulated by ISO ($P<0.05$); and plasma LDH activity were lower than that of ISO groups ($P<0.01$). Histological findings in taurine alone group were similar to the controls. Administration of taurine alone did not affect the myocardial MDA content and plasma LDH activity (Tab 1).

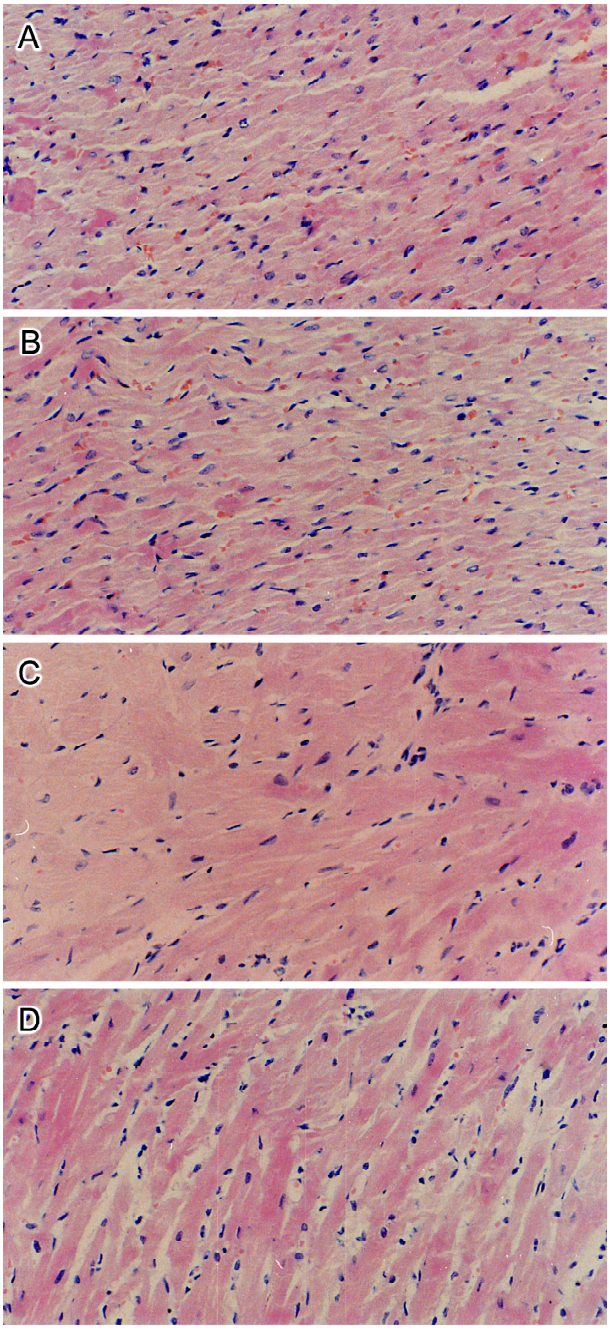

Fig 1. The myocardium was taken for pathological examination microscopically after hematoxylin-eosin stain. A: control sample; B: taurine-treated sample; C: ISO-treated sample; D: ISO+taurine-treated sample. ×400.

Heart and plasma taurine levels In ISO group, myocardial and plasma taurine contents reduced by 65 % ($P<0.01$) and increased by 49 % ($P<0.01$), respectively, as compared with those of control. In ISO+taurine group, however, both myocardial and plasma taurine levels were increased by 108 % and 370 % ($P<0.01$) , respectively, as compared with those of ISO (alone) group. In taurine (alone) group, plasma taurine content increased by 67 % ($P< 0.01$), but myocardial taurine content was not different with that of control group ($P>0.05$, Tab 1).

Myocardial [^{3}H]-taurine uptake and release The myocardial uptake amount of taurine in four groups increased with incubation time, and near to be saturated after 5 min. The uptake amount of the ISO group was obviously lower than that of the control group ($P<0.05$ or $P<0.01$). However, compared with ISO group, the taurine uptake in ISO plus taurine group was obviously augmented ($P<0.05$ or $P<0.01$). ISO treatment potentiated taurine release from myocardium. In ISO group, taurine release obviously increased by 45 % ($P<0.05$), 72 %, and 79 % ($P<0.01$) at 1, 2, and 5 min, respectively, as compared with that of the controls ($P<0.01$). Treatment with taurine attenuated markedly taurine release stimulated by ISO, the amount of taurine release in ISO plus taurine group was decreased by 18 % ($P>0.05$), 22 %, and 24 % ($P<0.05$) at 1, 2, and 5 min, respectively. Administration of taurine alone did not affect the myocardial uptake and release of taurine in normal rats (Fig 2).

[^{3}H]-Taurine bound to myocardial sarcolemma The activity of Na^+-K^+ ATPase (marker enzyme of sarcolemma) of membrane preparations from the left and right ventricular tissues were (6.6±0.6) and (6.2±0.6) $mmol \cdot h^{-1} \cdot g^{-1}$ protein, respectively, while in homogenates they were (0.97±0.11) and (0.94±0.13)

Tab 1. Effects of taurine on changes in myocardial contents of taurine, MDA, and LDH of rat hearts induced by isoproterenol. *n*=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. $^{e}P<0.05$, $^{f}P<0.01$ *vs* ISO-treated group.

Group	Heart coefficient/ mg·g^{-1}	Plasma LDH activity/U·L^{-1}	Myocardial MDA content/ μmol·g^{-1} protein	Plasma taurine content/μmol·L^{-1}	Myocardial taurine content/μmol·g^{-1} wet weight
Control	3.5±0.1	129±12	4.2±0.4	143±13	15±4
Taurine (2 %)	3.4±0.1	124±13	4.2±0.3	230±15^{b}	18±5
ISO (5 mg/kg)	5.4±0.3^{c}	308±11^{c}	6.0±0.5^{c}	213±14^{c}	5.0±1.5^{c}
ISO+taurine	4.8±0.1^{be}	176±8^{bf}	4.5±0.5^{e}	1002±14^{cf}	11.0±2.1^{b}

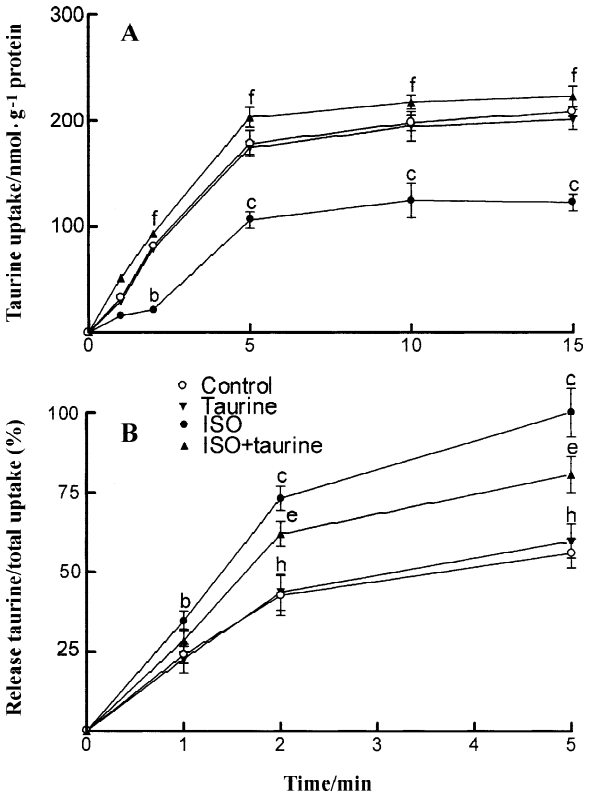

Fig 2. Effect of taurine incubation time on taurine uptake (A) and release and total taurine uptake percentage (B). *n*=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* ISO-treated group. $^{h}P<0.05$ *vs* ISO+taurine group.

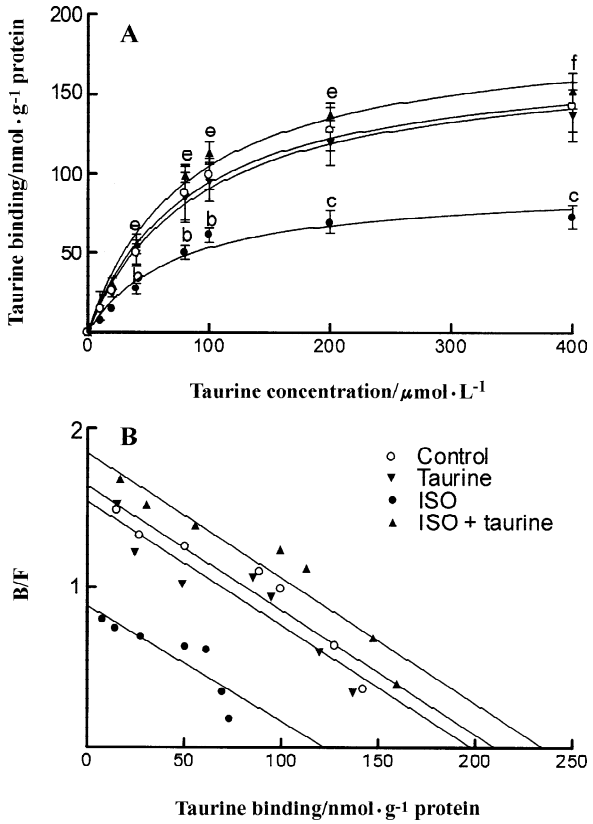

Fig 3. Myocardial sarcolemma bound taurine capacity at seven substrate concentrations (10, 20, 40, 80, 100, 200, and 400 mmol/L) from 6 independent experiments (A) and Scatchard plot of the data (B). *n*=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* ISO-treated group.

mmol·h^{-1}·g^{-1}, respectively. The activity of Na^+-K^+ ATPase of membrane preparations were 6.8 and 6.6 times of homogenate from left and right ventricle, respectively, indicating that membranes prepared were mainly sarcolemma. The course of concentration and binding of [^{3}H]-taurine for crude ventricular sarcolemma. Taurine bound to the sarcolemma with high affinity and in a concentration-dependent manner. In ISO group (Fig 3A), the maximal binding capacity (B_{max}) decreased by 42 %, as compared with that of control group (P<0.05). However, in ISO+taurine group it increased by 92 %, as compared with ISO group (P<0.01). Taurine alone did not affect taurine bound amount on sarcolemma. There were no statistical differences of K_d values among the four groups (P>0.05)

(Tab 2, Fig 3B).

Tab 2. Comparison of apparent taurine B_{max} and K_d of myocardial sarcolemma among the four groups. n=6. Mean±SD. ^cP<0.01 *vs* control group. ^fP<0.01 *vs* ISO-treated group.

Group	B_{max}/nmol·g^{-1} protein	K_d/μmol·L^{-1}
Control	210±5	129±9
Taurine (2 %)	198±13	129±10
ISO (5 mg/kg)	123±7^c	138±15
ISO+taurine	235±10^f	127±12

Myocardial TAUT mRNA content In this study, we used a competitive quantitative 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. In ISO group, myocardial TAUT mRNA was significantly reduced by 40 %, as compared with control group (P<0.05), whereas treatment with taurine attenuated suppressed effect of TAUT mRNA generation by ISO, TAUT mRNA content in ISO+taurine group was increased by 44 %, compared with ISO (alone) group (P<0.05). However, no significant change in the mRNA level was found between taurine (alone) and control groups (P>0.05, Fig 4).

Myocardial CSD mRNA content Administration of ISO (ISO group) decreased obviously myocardial CSD mRNA content by 38 % (P<0.05), as compared with that of control group. Treatment with taurine antagonized ISO-induced suppression of CSD expression, and myocardial CSD mRNA content in ISO plus taurine group was increased by 23 % (P<0.05), as compared with ISO (alone) group. However, no significant change in the mRNA level was found between taurine (alone) and control groups (Fig 5, P>0.05).

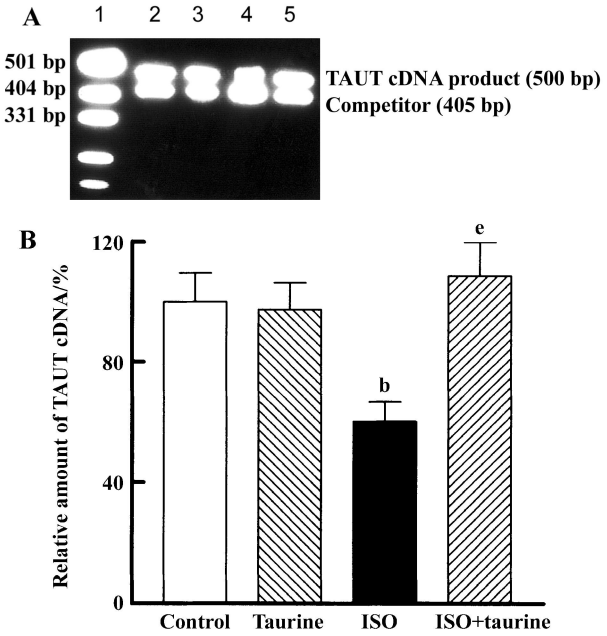

Fig 4. An example of a quantitative RT-PCR experiment. PCR products of TAUT cDNA were separated in a 1.5 % agarose gel. Lane 1, standand of DNA molecular weight; lane 2, control; lane 3, taurine-treated sample; lane 4, ISO-treated sample; lane 5, ISO+taurine-treated sample (A). Measurement of myocardial TAUT cDNA by quantitative RT-PCR from 6 independent experiments. Mean±SD. ^bP<0.05 vs control group. ^eP<0.05 *vs* ISO-treated group (B).

DISCUSSION

Taurine is most abundant free amino acid in tissues that are excitable such as nerve and muscle. Taurine can be accumulated in via two mechanisms: transport from the extracellular space and biosynthesis within the cell. Intracellular taurine maintains extraordinarily high concentration gradients across the intra- and the extra-cellular spaces through taurine transporter function[1]. For the rat heart, the taurine gradient of 200:1 is maintained. Taurine can be synthesized from its amino acid precursor, methionine and cysteine by metabolic mechanisms. 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[2]. Animals such as humans, cats, and certain monkeys that are unable to synthesize meaningful quantities of taurine, must rely on dietary sources of taurine to maintain their body loads. Taurine transport at the level of the brush border membrane in renal proximal tubules was regulated by circulating taurine and maintained the taurine body pool through regulating renal reabsorption[1,2]. Kidney maintains its metabolic balance in the body. Many functions of taurine, such as membrane stabilization, detoxification, antioxidant, osmoregulatory, calcium modulator, and acting as an inhibitory neurotransmitter or a neuromodulator, have been proposed. Transport of taurine across cell membranes is one of the major steps to play its physiological roles. For example, the biophysical and biochemial properties of taurine make it an excellent candidate for osmoregulation. During hyperosmolar conditions the

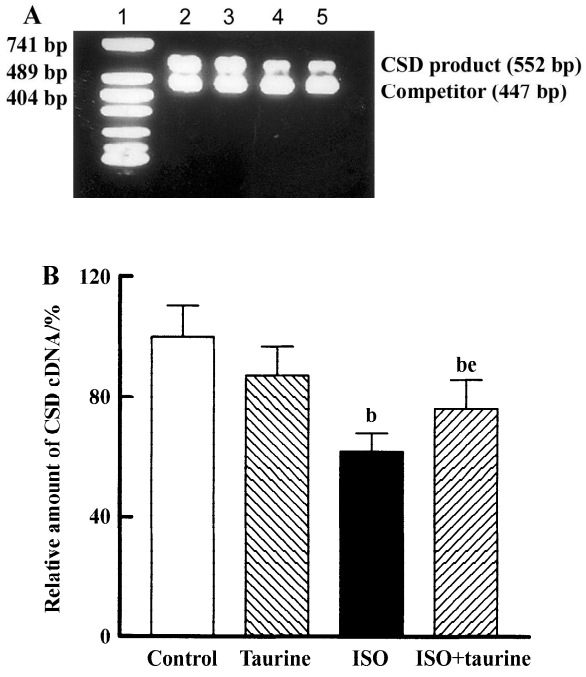

Fig 5. An example of a quantitative RT-PCR experiment. PCR products of CSD cDNA were separated in a 1.5 % agarose gel. Lane 1, standand of DNA molecular weight; lane 2, control; lane 3, taurine-treated sample; lane 4, ISO-treated sample; lane 5, ISO+taurine-treated sample (A). Measurement of myocardial CSD cDNA by quantitative RT-PCR from 6 independent experiments. Mean±SD. $^{b}P<0.05$ *vs* control group. $^{c}P<0.05$ *vs* ISO-treated group (B).

increase in cell taurine content appears to be achieved primarily by active transport of taurine into the cell; while under hyposmolar conditions decrease in intracellular taurine content is a consequence of a marked stimulation in taurine efflux, which prevents cells from dehydration or edema[12]. Taurine may also be a Ca^{2+} modulator. Both low and high extracellular Ca^{2+} concentration can lead to the changes of taurine transport. Taurine has the dual ability to increase Ca^{2+} availability under conditions of low Ca^{2+} concentration, but to protect against Ca^{2+} overload under conditions of high Ca^{2+} concentration[13].

Repeated injection of small doses of ISO results in subacute impairment of heart, such as infarction-like necrosis, massive fibrosis, compensatory cardiomegaly, increase of cytosolic enzyme LDH leaking into the extracellular space, and promoting lipid peroxidation[3]. Taurine protects against oxidative damage under many conditions, decreasing MDA formation and augmenting membrane-stabilizing actions, ie, it reduces the consequence of membrane damage. In this study, we also found that taurine was apparently beneficial for the ISO-induced myocardial injury in rats. In ISO group, myocardial taurine content was significantly reduced with increasing plasma taurine level, suggesting the decrease of taurine uptake by myocardium, and/or the increase of taurine release out of myocardium. Experiment of incubated myocardial tissue also showed that ISO treatment induced dysfunction of taurine transport, which resulted in the decrease of taurine content in myocardial tissue due to the impairment of active uptake of taurine against taurine gradient and the increased release of taurine from the tissue. Binding of taurine to TAUT is the first and important step, which taurine is accumulated intracellular space. In this study, we found that myocardial B_{max} of [^{3}H]-taurine binding was significantly reduced without change of the K_d value in ISO-treated rats, indicating that the decreased number of binding sites on cell membrane may be the major cause for the dysfunction of taurine transport. In ISO group, myocardial TAUT mRNA content reduced markedly, it suggested that dysfunction of taurine transport was relative to the down-regulation of TAUT gene expression. Therapy with taurine attenuated obviously ISO-induced cardiac impairment, with improving TAUT gene expression and function, and increasing myocardial taurine content. The mechanism of taurine cardioprotection effects could be considered the up-regulation of TAUT gene expression on myocardial sarcolemma. Administration of taurine to normal rats did not affect myocardial TAUT and CSD mRNA contents and its function. Considering taurine biosynthesis, it was found that treatment with ISO lowered myocardial CSD mRNA content, indicating that taurine biosynthesis reduced in ISO-injured myocardium. Therapy with taurine alleviated significantly ISO-induced suppression of CSD mRNA generation.

High concentration of taurine within cells is maintained by a combination of TAUT activity and endogenous synthesis. Taurine biosynthetic activity is low in humans, and so in most tissues taurine is actively taken up via unidirectional and Na^+-dependent transport. Nonetheless, the characteristic regulation of myocardial TAUT expression and function is still not well known[14]. cDNAs for the TAUT have been cloned, and sequence homology places it within the gene family of Na^+- and/or Cl^--dependent neurotransmitter transporters. TAUT cDNAs from rats encodes proteins of 621 amino acids exhibiting 12 putative transmembrane segments and multiple potential PKC and protein kinase A (PKA) phosphorylation sites. Changes in signal trans-

duction pathways could potentially play an important role in the overall effect of TAUT activity. TAUT activity is known to be differentially regulated by PKC, PKA, and Ca^{2+}/calmodulin-dependent protein kinases (CaMK). PKC activation has been reported to inhibit TAUT activity. The role of PKA in mediating the effects is, however, unclear, as both PKA activation and inhibition decreased TAUT activity. Because the TAUT has multiple potential PKA (and PKC) phosphorylation sites, the seeming paradoxical effects of PKA on TAUT activity may reflect differential responses to PKA-mediated phosphorylation at different sites on the TAUT protein. A critical site for phosphorylation by PKC was found in taurine transporter located at serine-322 in the fourth intracellular domain. Phosphorylation of this site will inhibit the activity of taurine transporter. Catecholamine, IL-1, and angiotensin II have been known to be the strong activators of PKC. ISO-induced cardiac hypertrophy and injury is the consequence of hypertrophic stimulus-induced changes in gene expression, which is linked by intracelluar PKA and PKC signal transduction pathways. Further study should be conducted on the relationship between taurine transport inhibition in ISO-induced myocardial injury and changes in intracellular signaling of PKA, PKC, and CaMK.

CSD is a 2396 bp cDNA sequence, which is encoding a protein of 493 amino acids (calculated molecular mass, 55.2 kDa)[15]. CSD belongs to a family of pyridoxal 5'-phosphate (PLP)-dependent enzymes, all of which decarboxylate a-amino acids to form the corresponding amines. The expression of CSD was reported to be regulated by adrenal hormones. CSD expression was downregulated by ISO, and its mechanism is still unclear. Further study should be conducted on the relationship between CSD inhibition in ISO-induced myocardial injury and changes of hormones.

Taurine is an ample substance in the body, and it should have no toxicity and drug dependence and tolerance with very wide range of safety when used clinically. Further studies should therefore be made in its clinical applications as a reagent and on the taurine transport in cardiovascular diseases, which contributed to elucidate the mechanisms of taurine transport dysfunction and might find some new strategies for the prevention and therapy of diseases.

REFERENCES

1 Huxtable RJ. Physiological action of taurine. Physiol Rev 1992; 72: 101-46.

2 Reymond I, Bitoun M, Levillain O, Tappaz M. Regional expression and histological localization of cysteine sulfinate decarboxylase mRNA in the rat kidney. Histochem Cytochem 2000; 48: 1461-8.

3 Ohta H, Azuma J, Awata N, Hamaguchi T, Tanaka Y, Sawamura A, *et al*. Mechanism of the protective action of taurine against isoprenaline induced myoocardial damage. Cardiovasc Res 1988; 22: 407-13.

4 Keep RF, Xiang J. Choroid plexus taurine transport. Brain Res 1996; 715: 17-24.

5 Gu J, Li XJ, Sun FY. Melatonin decreases the release of amino acid transmitters in hypoxic rat cortical slice. Acta Physiol Sin 1999; 51: 445-8.

6 Wolff AA, Hines DK, Karliner JS. Refined membrane preoarations mask ischemic fall in myocadial beta-receptor density. Am J Physiol 1989; 257: H1032-6.

7 Tang C, Hsu HK, Chen XY, Liu MS. Externalization and internalization of (Na^+ K^+)-ATPase in rat heart during different phases of sepsis. Circ Shock 1993; 41: 19-25.

8 Askawa T, Matashushita S. Thiobarbituric acid test for detecting lipid peroxide. Lipids 1979; 14: 401-6.

9 Paul DS, James WL. Competitive PCR. Nature 1992; 359: 557-8.

10 Celi FS, Zenilman ME, Shuldiner AR. A rapid and versatile method to synthesine internal standards for competitive PCR. Nucleic Acids Res 1993; 21: 1047.

11 Tsai SJ, Miltbank MC. Standard curve quantitative competitive RT-PCR (SC-QC-RT-PCR): a simple method to quantify absolute concentration of mRNA from limited amounts of sample. In: Siebert PD, Larrick JW, editors. Gene Cloning and analysis by RT-PCR. Natick MA: Biotechniques Books; 1998. p 91-101.

12 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 2001; 442: 87-95.

13 Militante JD, Lombardini JB. Stabilization of calcium uptake in rat rod outer segments by taurine and ATP. Amino Acids 2000; 19: 561-70.

14 Takeuchi K, Toyohara H, Sakaguchi M. A hyperosmotic stress-induced mRNA of carp cell encodes Na^+ and Cl^--dependent high affinity taurine transporter. Biochim Biophys Acta 2000; 1464: 219-30.

15 Tappaz M, Bitoun M, Reymond I, Sergeat A. Characterization of the cDNA coding for rat brain cysteine sulfinate decarboxylase: brain and liver enzymes are identical proteins encoded by teo distinct mRNAs. J Neurochem 1999; 73: 903-12.