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ORIGINAL ARTICLE

CSD mRNA expression in rat testis and the effect of taurine on testosterone secretion

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Abstract In the present study, the cysteine sulfinate decarboxylase (CSD) mRNA expression was detected in rat testis by RT-PCR. The results showed that CSD mRNA was expressed in rat testis, and the putative encoded-amino acid sequence was exactly the same as that in rat liver which was already known. At the same time, the effects of taurine on testosterone secretion were investigated both in vivo and in vitro. In vivo, taurine were administered to male rats by tap water. The results showed that taurine obviously stimulated the secretion of FSH, LH and testosterone in serum, but showed no significant effect on the secretion of estradiol. Taurine administered in water could significantly increase the concentration of taurine in the blood and testis of rats. In vitro, cultured Leydig cells were treated with taurine independently or incubated with human chorionic gonadotropin (HCG) and progesterone. The results showed that taurine had biphasic effects on basal testosterone secretion in cultured Leydig cells. Low concentrations of taurine (0.1–100 μg/ml) could stimulate testosterone secretion, whereas high concentration of taurine (400 μg/ml) could inhibit testosterone secretion. Testosterone secretion stimulated by HCG was significantly increased by 10 and 100 μg/ml of taurine administration, and obviously decreased by treating with 400 μg/ml of taurine. Testosterone secretion induced by progesterone was significantly stimulated by treating with 1.0 and 10 μg/ml of taurine, however, it was significantly inhibited when treated with 400 μg/ml of taurine. Meanwhile, the effect of silencing CSD mRNA by siRNA on testosterone secretion was analyzed. The results showed that testosterone secretion was obviously decreased after the inhibition of CSD mRNA expression in cultured Leydig cells. These results indicated that taurine can be synthesized in rat testis by CSD pathway, and it plays important roles in testosterone secretion both in vivo and in vitro which need to be further investigated.

Keywords Taurine · CSD mRNA expression · Testosterone secretion · Leydig cell · Rat

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Introduction

Taurine (2-aminoethansulfonic acid), first isolated in 1827 from ox-bile, is one of the end products of sulfur metabolism (Tiedemann and Gmelin 1827). It has been demonstrated to be a potentially nutritionally important amino acid in animals and human, which involved in a variety of functions, such as a neurotransmitter or neuroregulator (Kuriyama et al. 1978; Bligh 1981), bile formation in the liver (Vessey 1978), osmoregulation (Lasserre and Gilles 1971), modulation of calcium levels (Timbrell et al. 1995), stabilization of membranes (Huxtable and Bressler 1973), antiarrhythmic activity in the heart (Read and Welty 1963) and essential roles in the normal development of the brain (Magnusson 1994).

In the male reproductive system, taurine has been detected in Leydig cells, vascular endothelial cells, and other interstitial cells of testis, epithelial cells of the efferent ducts by immunohistochemical methods (Lobo et al. 2000), and was found to be one of the most abundant free amino acids in the germen, germ cell and seminal fluid (Velazquez et al. 1986; Holmes et al. 1992). Taurine may act as an

antioxidant in preventing sperm lipid peroxidation (Alvarez and Storey 1983), a capacitating agent (Meizel 1985), and a sperm motility factor (Boatman et al. 1990). It has been suggested that taurine inhibits plasma membrane Na^+, K^+-ATPase in membrane homogenates from hamster epididymal sperm cells (Mrsny and Meizel 1985). Taurine could also modify sperm phospholipids methyltransferase activity (Llanos and Ronco 1994).

The major synthetic site for taurine is thought to be the liver in most animals. Several possible pathways have been proposed for taurine synthesis, but the most widely accepted pathway is cysteine sulphinic acid (CSA) pathway, and cysteine sulfinate decarboxylase (CSD) is considered to be the main rate-limiting enzyme for taurine synthesis. The enzymatic activity of CSD was first identified in the liver (Hope 1955) and the CSD cDNA with approximately 1,800 bp length was generated from liver ploy(A)$^+$ mRNA (Isabelle et al. 1996). Recent work, however has shown that CSD mRNA are expressed in many extrahepatic tissues (Oertel et al. 1981; Pasantes-Morales et al. 1976; Hu et al. 2000; Li et al. 2006), suggesting that the taurine biosynthetic pathway may play an important role in nonhepatic tissues. The testis is one of particular interest because of importance of taurine in the male reproduction. But there is no essential available information about CSD expression in testis and the effect of taurine on testosterone secretion except for a single report of CSD mRNA expression in mice testis (Li et al. 2006).

In the present study, to determine whether taurine biosynthetic pathway is expressed in rat testis, and whether taurine influences testosterone secretion, the expression of CSD mRNA was investigated in rat testis by means of RT-PCR, at the same time, the effects of taurine on testosterone secretion in vivo and in vitro were also investigated.

Methods and materials

Experimental animals

Male Wistar rats were obtained from the central animal house of Chinese Medical University. They were maintained in controlled light (14 h-light, 10 h-dark) and temperature (22 ± 2°C), and given free access to rat chow and water.

Extraction of total RNA

The rats were sacrificed by decapitation at the end of 3rd, 10th and 78th week after birth. The testis and liver were immediately dissected out and frozen in liquid nitrogen, and stored at −80°C until RNA analysis. Total RNA was extracted by RNAiso Reagent and purified by DNase I (RNase Free) according to the procedure of the supplier (TaKaRa, China). The concentration and quality of total RNA were analyzed by spectrophotometry at 260 and 280 nm.

RT-PCR for testis CSD mRNA

The primers were designed by means of the Oligo6.0 system based on the sequence of rat CSD cDNA reported previously (Isabelle et al. 1996).The forward primer, 5′-GATGGC TGACTCAAAACCAC-3′ is from position 186–205(20 bp) and the reverse primer, 5′-GTTAGAGATGGAACCAC CAG-3′, is from position 635–654(20 bp).The predicted size of RT-PCR product using above two primers is 468 bp.

The testis and liver mRNA RT-PCR were performed using BcaBESTTM RNA PCR Kit (TaKaRa, China) in the thermal cycler (Nippon Ferrofluidics Corp., Hangzhou Dahe Thermo-Magnetics Co. Ltd., China) for 33 or 35 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, and the final extension at 72°C for 10 min.

After PCR, 5 μl of the reaction mixture was electrophoresed on a 1% agarose gel in 1 × TAE buffer and the bands were detected by ethydium bromide (2 μl/ml) staining. After washing with dH_2O, the agarose gel was photographed by JEDA 801 Polaroid film (Jiangsu JEDA Science-Technology Development CO.LTD., China).

Sequence analysis of testis CSD cDNA

After photographed, a PCR product forming a band at approximately 468 bp was recovered, and cloned into the ligated pUCm-T Vector (TaKaRa, China). The cloned vector was transfered to E. coli JM109 competent cells, and the transformed cells were cloned and proliferated. The plasmid DNA was recovered from each transformant. The nucleotide sequence was determined with the dideoxy chain termination method by TaKaRa, China. The encoded-amino acid sequence of CSD cDNA obtained from rat testis was putatived with DNASIS 2.5 system, and the putative encoded-amino acid sequences were analyzed through BLAST 2 SEQUENCE system (http://www.ncbi.nlm.nih.gov).

Effects of taurine on reproductive hormone levels in serum

Two-month-old rats were randomly divided into four groups, six in each group. Rats in control group were given tap water, rats in the other three groups were given water containing 0.5, 1 and 1.5% taurine, respectively. The rats were euthanatized after treatment for 5 weeks, and then blood and testis tissues were collected. Blood were used for

follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E) and taurine analyses. Testis was used for analyzing the content of taurine by way of high performance liquid chromatography (HPLC). The concentration of FSH, LH, T and E was detected by radio immunoassay.

Effects of taurine on testosterone secretion in cultured Leydig cells

Leydig cells were isolated and purified according to the reported method (Dou and Li 1999). The substances for administration were dissolved in DMEM. Taurine was administered at different concentration gradients (0.1, 1.0, 10, 100, 400 μg/ml). Progesterone and HCG were administered with a final concentration of 30 ng/ml and 10 IU/ml, respectively. After incubation for 24 h in the CO_2 incubator at 37°C and 5% CO_2, the supernatant was collected for testosterone analysis. At the same time, siRNA (5′-CCGGUUCUUCAACCAGCUCUU-3′, synthesized by Biomics Biotechnologyies, China) screened by laboratory, which could inhibit the expression of CSD mRNA, and Pu6H1-GFP (Biomics Biotechnologyies, China) were ligated according to the introduction of the kits to construct siRNA vector, as well as constructing the negative control vector (siRNA-NC). Transfected plasmid 4 μg and 10 μl LipofectamineTM2000 (Invitrogen, US) were diluted with 250 μl serum-free medium, respectively, and then mixed after 5 min, incubated at room temperature for 25 min. Then leydig cells (2×10^5 cell/well)cultured in serum-free medium for 48 h were put into the above mixture, incubated at 37°C for 6 h in 5% CO_2, then changed to serum medium which was added with 10 μg/ml taurine, after incubating for 48 h, observed under fluorescence microscope and cells were selected the ones with the transfection efficiency of about 65% for testosterone analysis by the method of radio immunoassay.

Statistical analysis

Data were presented as the mean ± SE and significant differences were determined by Duncan's multiple range test using SPSS 12.0 statistical analysis software. *P* values less than 0.05 were considered significant.

Results

Expression of CSD mRNA in rat testis

As Fig. 1 illustrates, the PCR products at around the predicted size of 468 bp were obtained from rat liver and testis samples. The nucleotide sequence and the putative

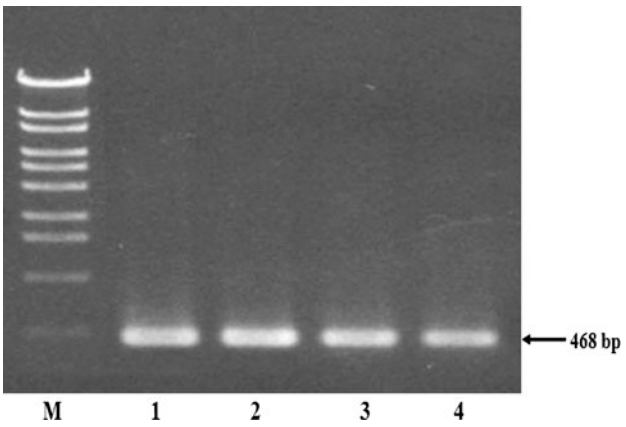

Fig. 1 Amplification of CSD mRNA by RT-PCR. Samples were obtained from the liver and the testis of rat. *M* marker. *1* liver CSD cDNA. *2–4* testis CSD cDNA (*2* 3 weeks old, *3* 10 weeks old, *4* 78 weeks old)

GATGGCTGACTCAAAACCACTCAGAACCCTGGATGGGGACCC
TGTGGCTGTGGAGGCTTTGCTCCGGGACGTGTTTGGGATTGT
CGTAGATGAGGCCATTCGGAAGGGGACCAATGCCTCTGAGAA
GGTCTGCGAATGGAAGGAGCCTGAAGAGCTCAAGCAGCTGCT
GGACTTGGAGCTGCAGAGCCAGGGCGAGTCTAGGGAGCGGA
TCCTGGAGCGCTGCCGGGCTGTGATTCATTACAGTGTCAAGAC
TGGTCACCCCCGGTTCTTCAACCAGCTCTTCTCAGGATTAGAT
CCCCATGCTCTGGCCGGGCGCATCATTACGGAGAGCCTCAAT
ACCAGCCAGTACACATATGAGATTGCCCCCGTGTTTGTGCTCA
TGGAAGAGGAGGTGCTGAAGAAACTCCGTGCCCTTGTGGGCT
GGAACACTGGGGATGGGGTCTTCTGTCCTGGTGGTTCCATCT
CTAA

Fig. 2 The nucleotide sequence of testis CSD cDNA

encoded-amino acid sequence of the PCR product from the testis were analyzed and are shown in Figs. 2 and 3. The sequence of amino acid coded by amplified CSD cDNA in testis was the same as that in rat liver (100%).

Effects of taurine on reproductive hormone levels in serum

As shown in Fig. 4, the levels of FSH, LH and testosterone in serum were obviously increased by taurine administration ($p < 0.05$), but the level of estradiol showed no significant changes ($p > 0.05$). As illustrated in Fig. 5, the concentration of taurine in the serum and testis was significantly increased by taurine administration ($p < 0.05$).

Effects of taurine on testosterone secretion in cultured Leydig cells

As shown in Fig. 6, the testosterone secretion was obviously stimulated by taurine administration at the range of

Fig. 3 The comparison of testis CSD cDNA amino acid sequence (Query) and that of liver CSD cDNA already reported (Sbjct)

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Score = 278 bits (710), Expect = 4e-74
Identities = 155/155 (100%), Positives = 155/155 (100%)
Query: 1 MADSKPLRTLDGDPVAVEALLRDVFGIVVDEAIRKGTNASEKVCEWKEPEELKQLLDLEL 60
         MADSKPLRTLDGDPVAVEALLRDVFGIVVDEAIRKGTNASEKVCEWKEPEELKQLLDLEL
Sbjct: 1 MADSKPLRTLDGDPVAVEALLRDVFGIVVDEAIRKGTNASEKVCEWKEPEELKQLLDLEL 60
Query: 61 QSQGESRERILERCRAVIHYSVKTGHPRFFNQLFSGLDPHALAGRIITESLNTSQYTYEI 120
          QSQGESRERILERCRAVIHYSVKTGHPRFFNQLFSGLDPHALAGRIITESLNTSQYTYEI
Sbjct: 61 QSQGESRERILERCRAVIHYSVKTGHPRFFNQLFSGLDPHALAGRIITESLNTSQYTYEI 120
Query: 121 APVFVLMEEEVLKKLRALVGWNTGDGVFCPGGSIS 155
           APVFVLMEEEVLKKLRALVGWNTGDGVFCPGGSIS
Sbjct: 121 APVFVLMEEEVLKKLRALVGWNTGDGVFCPGGSIS 155
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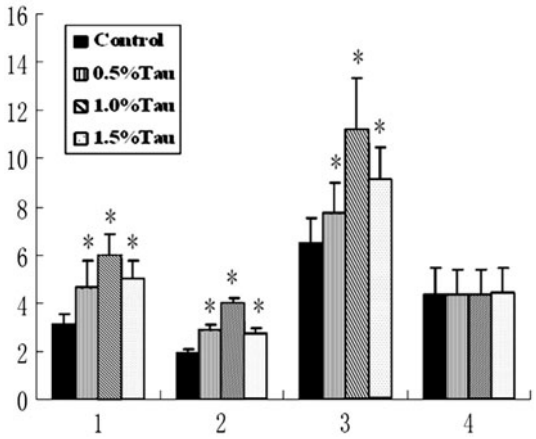

Fig. 4 Effects of taurine on the secretion of reproductive hormone in serum. *1* FSH (mIU/ml), *2* LH (IU/L), *3* T (ng/dl), *4* E_2 (pg/ml). Results are presented as mean ± SE ($n = 6$). *Significantly different from control group ($p < 0.05$)

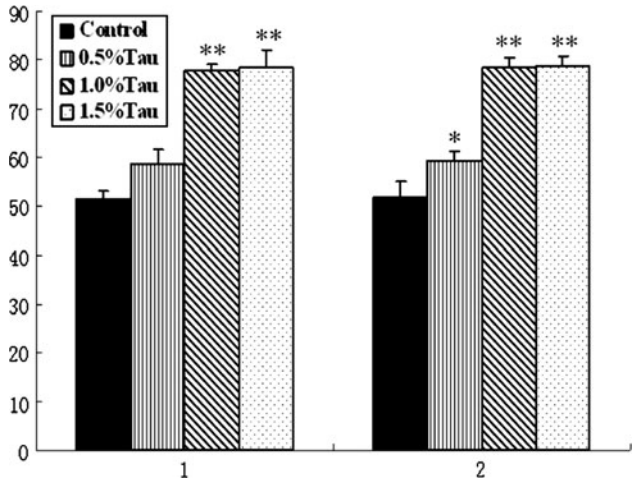

Fig. 5 The concentration of taurine in serum and testis. *1* Serum, *2* testis. Results are presented as mean ± SE ($n = 6$).*Significantly different from control group ($p < 0.05$), **Significantly different from control group ($p < 0.01$)

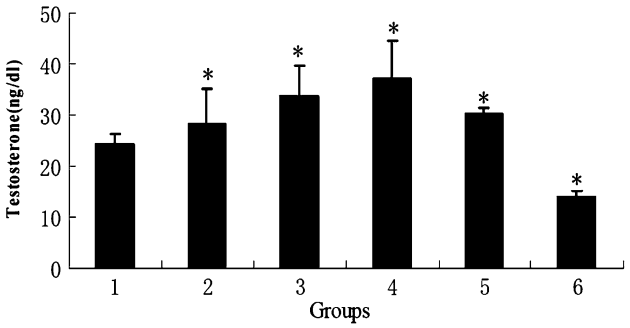

Fig. 6 Effects of taurine on basal testosterone secretion in cultured Leydig cells. *1* Control, *2* 0.1 μg/ml taurine, *3* 1.0 μg/ml taurine, *4* 10 μg/ml taurine, *5* 100 μg/ml taurine, *6* 400 μg/ml taurine. Results are presented as mean ± SE ($n = 5$). *Significantly different from control group ($p < 0.05$)

0.1–100 μg/ml ($p < 0.05$), but the secretion was significantly inhibited by high dose of taurine administration (400 μg/ml) ($p < 0.05$).

Effect of taurine on testosterone secretion stimulated by HCG in cultured Leydig cells is shown in Fig. 7. The testosterone secretion stimulated by HCG showed no significant changes by 0.1 and 1.0 μg/ml of taurine administration ($p > 0.05$), but was significantly increased by 10 and 100 μg/ml of taurine administration ($p < 0.05$), and was obviously decreased by treating with 400 μg/ml of taurine ($p < 0.05$).

As shown in Fig. 8, testosterone secretion induced by progesterone was significantly stimulated by treating with 1.0 and 10 μg/ml of taurine ($p < 0.05$). However, testosterone secretion induced by progesterone was significantly inhibited when treated with 400 μg/ml of taurine ($p < 0.05$).

The effect of CSD mRNA interference on testosterone secretion in cultured Leydig cells is shown in Fig. 9. It was demonstrated that testosterone secretion was obviously decreased after the inhibition of CSD mRNA expression ($p < 0.01$). While, taurine could significantly stimulate testosterone secretion in RNAi Leydig cells ($p < 0.01$).

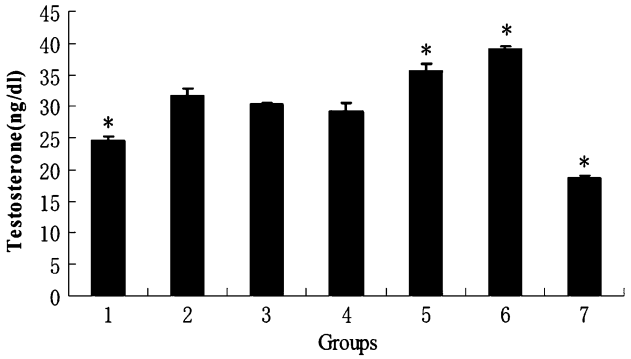

Fig. 7 Effects of taurine on testosterone secretion stimulated by HCG in cultured Leydig cells. *1* Control, *2* 10 IU HCG, *3* 10 IU HCG + 0.1 μg/ml taurine, *4* 10 IU HCG + 1.0 μg/ml taurine, *5* 10 IU HCG + 10 μg/ml taurine, *6* 10 IU HCG + 100 μg/ml taurine, *7* 10 IU HCG + 400 μg/ml taurine. Results are presented as mean ± SE ($n = 5$). *Significantly different from 10 IU HCG group ($p < 0.05$)

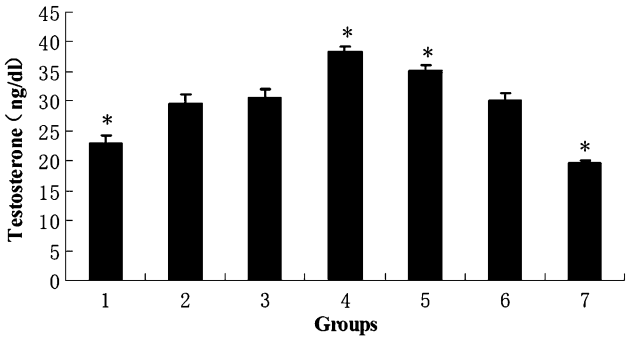

Fig. 8 Effects of taurine on testosterone secretion induced by progesterone in cultured Leydig cells. *1* Control, *2* 30 ng progesterone, *3* 30 ng progesterone + 0.1 μg/ml taurine, *4* 30 ng progesterone + 1.0 μg/ml taurine, *5* 30 ng progesterone + 10 μg/ml taurine, *6* 30 ng progesterone + 100 μg/ml taurine, *7* 30 ng progesterone + 400 μg/ml taurine. Results are presented as mean ± SE ($n = 5$). *Significantly different from 30 ng progesterone group ($p < 0.05$)

Discussion

The concentration of taurine is extremely high in mammalian germen, sperm and seminal fluid, and it has been reported that taurine has many effects on male reproduction (Fraser 1986; Alvarez and Storey 1983). However, where the high concentrations of taurine in the reproductive organs come from is unknown. Although there is considerable variation in the activity of CSD among different species, CSD is considered to be directly responsible for the synthesis of taurine. In rat, the liver is thought to be the major site of taurine biosynthesis (Hope 1955). The synthesis of taurine has also been reported in other tissues of the rat (Park et al. 2002). Biosynthesis of taurine by these extra-hepatic tissues may be essential for their own tissue.

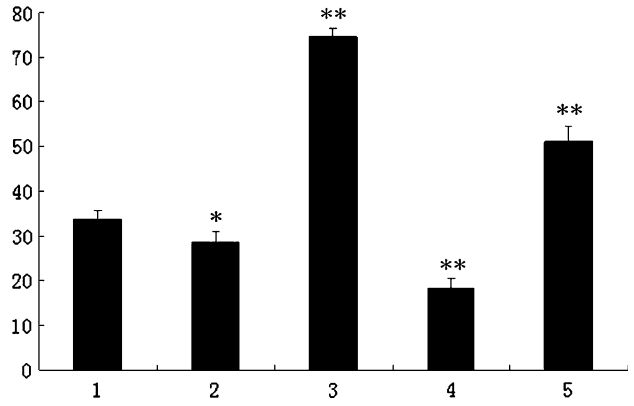

Fig. 9 Effects of RNA interference on testosterone secretion in cultured Leydig cells. *1* Control, *2* RNAi-NC, *3* 10 μg/ml taurine, *4* RNAi, *5* RNAi + 10 μg/ml taurine. Results are presented as mean ± SE ($n = 5$). *Significantly different from control group ($p < 0.05$), **Significantly different from control group ($p < 0.01$)

In the present study, the expression of CSD mRNA was confirmed in rat testis by RT-PCR, and the putative encoded-amino acid sequence was exactly the same as that in the liver. This made us conclude that the molecular species of CSD mRNA in rat testis is the same as that in the liver. The results suggested that taurine can also be synthesized in testis by CSD pathway, and high concentrations of taurine in the germen and seminal fluid have at least partially resulted from synthesis in testis.

The testis is responsible for the production of testosterone and the spermatogenesis. These two functions occur in the Leydig cells and seminiferous tubules, respectively. The biosynthesis of taurine in rat testis suggested that taurine may be essential for testicular function. To confirm whether taurine can influence the function of testis, the in vivo and in vitro experiments were conducted.

The in vivo results showed that taurine could stimulate the secretion of FSH, LH and testosterone in serum significantly. FSH, LH and testosterone were essential for male reproduction. It has been proved by our previous research that 1% beta-alanine added in water could significantly reduce serum testosterone of rats (Yang et al. 2007). In addition, our unpublished results showed that taurine could promote the motility and survival rate of sperm in old rats. All these results indicated that taurine may be important for the process of steroidogenesis and spermatogenesis.

The in vitro results showed that taurine had biphasic effects on basal testosterone secretion in cultured Leydig cells. Low concentrations of taurine could stimulate testosterone secretion, whereas high concentration of taurine could inhibit testosterone secretion, which may be a pharmacological action. The production of testosterone is regulated by many kinds of factors, such as LH/HCG, PRL,

GnRH, etc. Within the testis, LH/HCG binds to membrane receptors on the Leydig cells and stimulates them to convert cholesterol to testosterone. Progesterone is one of the most important precursors in testosterone synthesis. Our results demonstrated that taurine can significantly increase testosterone secretion stimulated by HCG and incubated with progesterone in cultured Leydig cells. The testosterone secretion was inhibited by CSD mRNA interference. The results indicated that taurine may be essential for testosterone secretion, and taurine may stimulate the testosterone secretion by way of increasing the sensitivity and the number of LH/HCG receptors on the Leydig cells. In addition, taurine may stimulate the testosterone secretion by increasing the enzyme activities in the production of testosterone, thus promoting the conversion from progesterone to testosterone.

Conclusion

In conclusion, our results showed that taurine can be synthesized in rat testis by CSD pathway, and it plays important roles in testosterone secretion both in vivo and in vitro, and its exact mechanisms need to be further investigated.

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