

The Synthesis and Role of Taurine in the Eel Spermatogenesis

Masato HIGUCHI, Fritzie T. CELINO, Chiemi MIURA and Takeshi MIURA

*South Ehime Fisheries Research Centre, Ehime University,
Funakoshi 1289-1, Ainan 798-4212, Japan*

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Abstract—Taurine has been shown to have various physiological functions in the body and its abundant in many organs, including brain, heart, kidneys and reproductive organs. Recently, we found that taurine synthesized in the Sertoli cells and, plays an important role in protection of germ cells from oxidative stress using *in vitro* the Japanese eel (*Anguilla japonica*) testicular organ culture system. We propose the new taurine function in spermatogenesis.

Keywords: taurine, progesterone, spermatogenesis, superoxide dismutase

INTRODUCTION

The male Japanese eel provides a superior system for studying the regulation of spermatogenesis because only spermatogonial stem cells are present in the testis when eel are in fresh water, and complete spermatogenesis can be induced, both *in vivo* by treatment with human chorionic gonadotropin (hCG), and *in vitro* by treatment with 11-ketotestosterone (KT), a major androgen in teleost fish (Miura and Miura, 2001). The series of our studies using this *in vitro* testicular organ culture system revealed that taurine plays an important role in testis.

Taurine (2-aminoethanesulfonic acid) is a free β -amino acid that is present at high concentrations in several eel tissues. In the eel testis, taurine is found mainly in the Sertoli cells and is weakly expressed also in spermatogonia. It has been believed that taurine is synthesized in the liver, however, recent studies indicate that taurine might be synthesized other organs such as brain, kidney and testis in mammals (Tappaz *et al.*, 1999; Park *et al.*, 2002; Li *et al.*, 2006). Furthermore, we found that the eel testis has ability to synthesize taurine. This is the new finding about taurine biosynthesis.

Taurine also acts as an antioxidant in preventing rabbit sperm lipid peroxidation (Alvarez and Storey, 1983), and taurine protects superoxide dismutase (SOD) activity from cadmium-induced or arsenic oxidative stress in mammals (Sinha *et al.*, 2008; Manna *et al.*, 2009; Das *et al.*, 2009). However, the preventive mechanism of action of taurine has not been fully cleared. We found that taurine promotes the total superoxide dismutase (SOD) activity in the eel testis. Moreover, we found that taurine does not affect the mRNA levels of copper-zinc SOD (Cu/

Zn SOD) or manganese SOD (Mn SOD), but promotes the translation of Cu/Zn SOD.

In this review, we focus on the synthesis of taurine and role in the protection germ cells from oxidative stress in the eel testis.

THE SYNTHESIS OF TAURINE IN EEL TESTIS

Taurine is synthesized either from the oxidation of L-cysteine via cysteine dioxygenase (CDO), which generates cysteine sulfinic acid that is decarboxylated by cysteinesulfinic acid decarboxylase (CSD). These reactions are essential for the biosynthesis of taurine. It has been postulated that taurine is mainly synthesized in the liver. However, it has been suggested that taurine is also synthesized in the brain (Pasantes-Morales *et al.*, 1980) and the testis (Yang *et al.*, 2010). Furthermore, recent studies have shown that both CDO and CSD are expressed in the mammary gland (Ueki and Stipanuk, 2007) and that CSD mRNAs are additionally expressed in the brain (Tappaz *et al.*, 1999), kidney (Park *et al.*, 2002), and testis (Li *et al.*, 2006). Recently, we have reported that both CDO and CSD are expressed in the Sertoli cells in the Japanese eel testis, and $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one (DHP), the fish progestin, promotes taurine synthesis in this organ via the up-regulation of CDO mRNA (Higuchi *et al.*, 2011). We also found that taurine exists mainly in the Sertoli cells and weakly expressed also in spermatogonia in Japanese eel. We have further found in the eel testis that taurine is actively accumulated via the sodium/chloride-dependent taurine transporter (TauT; SLC6A6) which is expressed in germ cells. Our current data indicate that taurine is synthesized in the Sertoli cells and transported into germ cells via the TauT.

The Sertoli cell has also been called as “nurse cell” because the Sertoli cell function largely to assist the developing spermatogonial germ cells through their maturation process. It is well known that Sertoli cell secretes numerous important hormones and other substances to trigger proper development of germ cell. Secretion of taurine is also to be involved in these functions of the Sertoli cell.

THE ROLE OF TAURINE IN THE PROTECTION OF GERM CELLS FROM OXIDATIVE STRESS

Taurine also acts as an antioxidant and protects against the toxicity of various substances *in vivo* (Alvarez and Storey, 1983; Boatman *et al.*, 1990; Green *et al.*, 1991; Güreş *et al.*, 2001; Balkan *et al.*, 2002). Recently also, it was reported that taurine protects the SOD pathway from cadmium-induced or arsenic oxidative stress (Sinha *et al.*, 2008; Manna *et al.*, 2009; Das *et al.*, 2009).

The SODs are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, which in turn is broken down to water by catalase in the peroxisomes, and by glutathione peroxidase in the cytosol and mitochondria. Three distinct types of SODs have been identified in mammals. Two isoforms have copper (Cu) and zinc (Zn) atoms in their catalytic center and are located either in the cytoplasm (Cu/Zn SOD) or in the extracellular elements (EC SOD). The third isoform has manganese (Mn SOD) as a cofactor and is located in the

mitochondria (Zelko *et al.*, 2002). Cells are normally protected against oxidative damage by multiple enzymatic mechanisms and by antioxidant molecules. The SODs are the first and most important line of antioxidant enzyme defense against oxidative stress, such as that caused by superoxide anion radicals.

We examined the *in vitro* effects of taurine on the SOD activity levels in the eel testis. Cultured testicular fragments exposed to taurine in the medium showed higher SOD activities than the control groups, and this effect was blocked by β -alanine, a competitive taurine transport inhibitor. It is also indicated that taurine does not affect the gene expression of either Cu/Zn SOD or Mn SOD, but induces the protein expression of Cu/Zn SOD only. It has been reported in this regard that several hormones such as prolactin, melatonin or growth hormone induce Cu/Zn SOD mRNA expression (Antolin *et al.*, 1996; Sugino *et al.*, 1998; Hauck and Bartke, 2000), and that the testicular 65 kDa protein specifically binds to the 5' UTR of testicular Cu/Zn SOD mRNA and represses its translation in mice (Gu and Hechet, 1996). However, there has been no report to date describing the up-regulation of Cu/Zn SOD protein synthesis. To our knowledge, our current study is the first to report that taurine increased the protein expression of Cu/Zn SOD in testis *in vitro*.

Oxidative stress has been implicated in germ cell apoptosis and DNA damage (Aitken and Krausz, 2001; Celino *et al.*, 2009; Paul *et al.*, 2009). From this perspective, taurine would play an important role in the protective mechanisms operating in germ cells by promoting the expression of Cu/Zn SOD in the cytosol of spermatogonia. Furthermore, it has been shown previously that Cu/Zn siRNA decreases the number of Cu/Zn SOD-positive spermatogonia and increases the level of oxidative damage, and also reported the strong expression of Cu/Zn SOD in spermatogonia, and low expression in spermatocytes, spermatids and spermatozoa (Celino *et al.*, 2011). These data collectively suggest that taurine is important for the protection of spermatogonia from oxidative stress.

The mechanisms by which taurine induces Cu/Zn SOD are unclear but three possibilities would exist. The first of these may involve the anti-oxidant properties of taurine. It is well known that taurine removes hypochlorous acid (HOCl) by reacting with it to form chlorotaurine (Timbrell *et al.*, 1995; Cunningham *et al.*, 1998), and that HOCl stress impairs SOD activity (Maalej *et al.*, 2006). However, sulfur-containing amino acids such as cysteine or methionine can also scavenge HOCl, and these amino acids are present at high levels in the L-15 culture medium used in the organ culture (cysteine; 0.99 mM, methionine; 0.5 mM) compared with the supplemented taurine (0.8–800 μ M) concentrations. It is therefore, difficult to favor this hypothesis.

The second possibility may be that taurine promotes Cu/Zn SOD protein synthesis. It has been reported that several amino acids such as leucine can individually activate signaling pathway to promote protein synthesis and possibly inhibit autophagy-mediated protein degradation (Anthony *et al.*, 2000; Rhoads and Wu, 2008). It was further found that small molecules directly bind to non-coding regions of mRNA and regulate gene expression, known as RNA switches or riboswitches (Winkler *et al.*, 2002; Rodionov *et al.*, 2003; Sudarsan *et al.*,

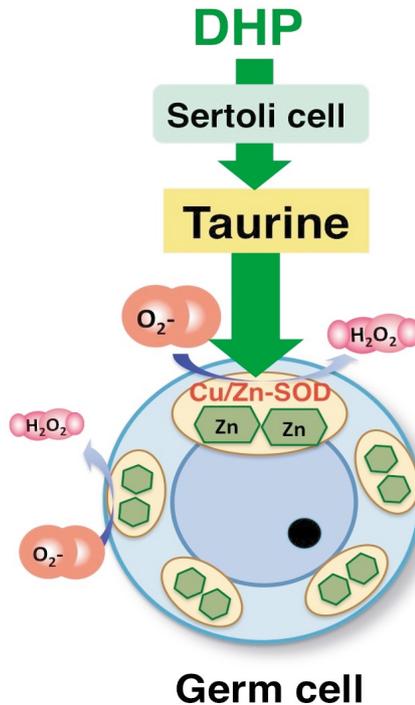


Fig. 1. Schematic illustration of the effect of taurine for protecting germ cell from oxidative stress by promoting Cu/Zn SOD expression. O_2^- , reactive oxygen species.

2003; Mandal *et al.*, 2004). These are mainly found in bacteria, fungi and plants, but were recently identified in human cells (Ray *et al.*, 2009). Moreover, two novel taurine-containing modified uridines are present in human and bovine mitochondrial tRNAs (Suzuki *et al.*, 2002). However, whether taurine can bind to mRNAs and modulate gene expression is not certain at present, and will require further study.

The third possibility may be an interaction between taurine and zinc. It is reported that taurine stimulates Zn(II) absorption in human fibroblasts and in rainbow trout intestine (Harraki *et al.*, 1994; Glover and Hogstrand, 2002). Moreover, Nusetti and co-workers have demonstrated that low zinc concentrations increase taurine uptake and high concentrations inhibit taurine transport (Nusetti *et al.*, 2010). We have also demonstrated previously that zinc modulates eel testicular Cu/Zn SOD activity (Celino *et al.*, 2011). To clarify this possible mechanism, it will be necessary in the future to analyze the interaction between taurine, Zn(II) and Cu/Zn SOD.

CONCLUSIONS

Taurine is synthesized in the Sertoli cells in the Japanese eel testis and, transported into germ cells via TauT. It is also suggested that taurine plays an important role in protection of germ cells from oxidative stress (Fig. 1).

REFERENCES

- Aitken, R. J. and C. G. Krausz (2001): Oxidative stress, DNA damage and the Y chromosome. *Reproduction*, **122**, 497–506.
- Alvarez, J. G. and B. T. Storey (1983): Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. *Biol. Reprod.*, **29**, 548–555.
- Anthony, J. C., F. Yoshizawa, T. G. Anthony, T. C. Vary, L. S. Jefferson and S. R. Kimball (2000): Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J. Nutr.*, **130**, 2413–2419.
- Antolin, I., C. Rodriguez and R. M. Sainz (1996): Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. *FASEB J.*, **10**, 882–890.
- Balkan, J., O. Kanbagli, G. Aykac-Toker and M. Uysal (2002): Taurine treatment reduces hepatic lipids and oxidative stress in chronically ethanol treated rats. *Biol. Pharm. Bull.*, **25**, 1231–1233.
- Boatman, D. E., D. B. Bavister and E. Cruz (1990): Addition of hypotaurine can reactivate immotile golden hamster spermatozoa. *J. Androl.*, **11**, 66–72.
- Celino, F. T., S. Yamaguchi, C. Miura and T. Miura (2009): Arsenic inhibits *in vitro* spermatogenesis and induces germ cell apoptosis in Japanese eel (*Anguilla japonica*). *Reproduction*, **138**, 279–287.
- Celino, F. T., S. Yamaguchi, C. Miura, T. Ohta, Y. Tozawa, T. Iwai and T. Miura (2011): Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. *PLoS ONE*, **6**(2), e16938, doi:10.1371/journal.pone.0016938.
- Cunningham, C., K. F. Tipton and H. B. F. Dixon (1998): Conversion of taurine into N-chlorotaurine (taurine chloramine) and sulphoacetaldehyde in response to oxidative stress. *Biochem. J.*, **330**, 939–945.
- Das, J., J. Ghosh, P. Manna, M. Sinha and P. C. Sil (2009): Taurine protects rat testes against NaAsO₂-induced oxidative stress and apoptosis via mitochondrial dependent and independent pathways. *Toxicol. Lett.*, **187**, 201–210.
- Glover, C. N. and C. Hogstrand (2002): Amino acid modulation of *in vivo* intestinal zinc absorption in freshwater rainbow trout. *J. Exp. Biol.*, **205**, 151–158.
- Green, T. R., J. H. Fellman, A. L. Eicher and K. L. Pratt (1991): Antioxidant role and subcellular location of hypotaurine and taurine in human neutrophils. *Biochim. Biophys. Acta*, **1073**(1), 91–97.
- Gu, W. and N. B. Hechet (1996): Translation of a testis-specific Cu/Zn SOD superoxide dismutase (SOD-1) mRNA is regulated by a 65-kilodalton protein which binds to its 5' untranslated region. *Mol. Cell. Biol.*, **16**, 4534–4543.
- Gürer, H., H. Ozgünes, E. Saygin and N. Ercal (2001): Antioxidant effect of taurine against lead-induced oxidative stress. *Arch. Environ. Contam. Toxicol.*, **41**(4), 397–402.
- Harraki, B., P. Guiraud, M. H. Rochat, H. Faure, M. J. Richard, M. Fusselier and A. Favier (1994): Effect of taurine, L-glutamine and L-histidine addition in an amino acid glucose solution on the cellular bioavailability of zinc. *BioMetals*, **7**, 237–243.
- Hauck, S. J. and A. Bartke (2000): Effects of growth hormone on hypothalamic catalase and Cu/Zn superoxide dismutase. *Free Radic. Biol. Med.*, **28**, 970–978.
- Higuchi, M., F. T. Celino, A. Tamai, C. Miura and T. Miura (2011): The synthesis and role of taurine in the Japanese eel testis. *Amino Acids*, doi:10.1007/s00726-011-1128-3.
- Li, J. H., Y. Q. Ling, J. J. Fan, X. P. Zhang and S. Cui (2006): Expression of cysteine sulfinatase decarboxylase (CSD) in male reproductive organs of mice. *Histochem. Cell Biol.*, **125**, 607–613.
- Maalej, S., I. Dammak and S. Dukan (2006): The impairment of superoxide dismutase coordinates

- the derepression of the PerR regulon in the response of *Staphylococcus aureus* to HOCL stress. *Microbiol.* **152**, 855–861.
- Mandal, M., M. Lee, J. E. Barrick, Z. Weinberg, G. M. Emilsson, W. L. Ruzzo and R. R. Breaker (2004): A glycine-dependent riboswitch that uses cooperative binding to control gene expression. *Science*, **306**, 275–279.
- Manna, P., M. Sinha and P. C. Sil (2009): Taurine plays a beneficial role against cadmium-induced oxidative renal dysfunction. *Amino Acids*, **36**, 417–428.
- Miura, T. and C. Miura (2001): Japanese eel: a model for analysis of spermatogenesis. *Zool Sci.*, **18**(8), 1055–1063.
- Nusetti, S., M. Urbina, F. Obregon, M. Quintal, Z. Benzo and L. Lima (2010): Effects of zinc *ex vivo* and intracellular zinc chelator *in vivo* on taurine uptake in goldfish retina. *Amino Acids*, **38**, 1429–1437.
- Park, E., S. Y. Park, C. Wang, J. Xu, G. LaFauci and G. Schuller-Levis (2002): Cloning of murine cysteine sulfenic acid decarboxylase and its mRNA expression in murine tissues. *Biochim. Biophys. Acta*, **1574**, 403–406.
- Pasantes-Morales, H., F. Chatagner and P. Mandel (1980): Synthesis of taurine in rat liver and brain *in vivo*. *Neurochem. Res.*, **5**, 441–451.
- Paul, C., S. Teng and P. T. K. Saunders (2009): A single, mild transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. *Biol. Reprod.*, **80**, 913–919.
- Ray, P. S., J. Jia, M. Majumder, M. Hatzoglou and P. L. Fox (2009): A stress-responsive RNA switch regulates VEGFA expression. *Nature*, **457**, 915–919.
- Rhoads, J. M. and G. Wu (2008): Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids*, doi:10.1007/s00726-008-0225-4.
- Rodionov, D. A., A. G. Vitreschak, A. A. Mironov and M. S. Gelfand (2003): Regulation of lysine biosynthesis and transport genes in bacteria: yet another RNA riboswitch? *Nucleic Acids Res.* **31**, 6748–6757.
- Sinha, M., P. Manna and P. C. Sil (2008): Taurine protects the antioxidant defense system in the erythrocytes of cadmium treated mice. *BMB Reports Sep.* **30**, **41**(9), 657–663.
- Sudarsan, N., J. K. Wickiser, S. Nakamura, M. S. Ebert and R. R. Breaker (2003): An mRNA structure in bacteria that controls gene expression by binding lysine. *Genes Dev.*, **17**, 2688–2697.
- Sugino, N., M. Hiroswawa-Takamori and L. Zhong (1998): Hormonal regulation of copper-zinc superoxide dismutase and manganese superoxide dismutase messenger ribonucleic acid in the rat corpus luteum: induction by prolactin and placental lactogens. *Biol. Rept.*, **59**, 599–605.
- Suzuki, T., T. Suzuki, T. Wada, K. Saigo and K. Watanabe (2002): Taurine as a constituent of mitochondrial tRNA: new insights into the functions of taurine and human mitochondrial diseases. *EMBO J.*, **21**, 6581–6589.
- Tappaz, M., M. Bitoun, I. Reymond and A. Sergeant (1999): 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.
- Timbrell, J. A., V. Seabra and C. J. Watereld (1995): The *in vivo* and *in vitro* protective properties of taurine. *Gen. Pharmacol.*, **26**, 453–462.
- Ueki, I. and M. H. Stipanuk (2007): Enzymes of the taurine biosynthetic pathway are expressed in rat mammary gland. *J. Nutr.*, **137**, 1887–1894.
- Winkler, W. C., S. Cohen-Chalamish and R. R. Breaker (2002): An mRNA structure that controls gene expression by binding FMN. *Proc. Natl Acad. Sci. USA*, **99**, 15908–15913.
- Yang, J. C., G. F. Wu, Y. Feng, C. M. Sun, S. M. Lin and J. M. Hu (2010): CSD mRNA expression in rat testis and the effect of taurine on testosterone secretion. *Amino Acids*, **39**(1), 155–160.
- Zelko, I., T. J. Mariani and R. J. Folz (2002): Superoxide dismutase multi-gene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radical. Biol. Med.*, **33**, 337–349.