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ORIGINAL ARTICLE

The synthesis and role of taurine in the Japanese eel testis

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Abstract In teleost fish, the progestin 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) is an essential component of the spermatogenesis pathway. In a series of investigations on the mechanisms underlying progestin-stimulated spermatogenesis, we have found that DHP up-regulates the expression of cysteine dioxygenase1 (CDO1) in the Japanese eel testis. CDO1 is one of the enzymes involved in the taurine biosynthesis pathway. To evaluate whether taurine is synthesized in the eel testis, cysteine sulfinate decarboxylase (CSD), another enzyme involved in taurine synthesis, was isolated from this species. RT-PCR and in vitro eel testicular culture revealed that although CSD was also expressed in eel testis, neither DHP nor other sex steroids affect CSD mRNA expression in a similar manner to CDO1. Using an in vitro eel testicular culture system, we further investigated the effects of DHP on taurine synthesis in the eel testis. HPLC analysis showed that DHP treatment significantly increases the taurine levels in the eel testis. These results suggest that DHP promotes taurine synthesis via the up-regulation of CDO1 mRNA expression during eel spermatogenesis. Furthermore, we observed from our analysis that although taurine does not induce complete spermatogenesis, it promotes spermatogonial DNA synthesis and the expression of Spo11, a meiosis-specific marker. These data thus suggest that taurine augments the effects of sex steroids in the promotion of spermatogonial proliferation and/or meiosis and hence that taurine plays important roles in spermatogenesis.

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Introduction

Spermatogenesis is controlled by various hormones and other factors. The Japanese eel (*Anguilla japonica*) is an ideal model for analyzing this process because spermatogenesis in the eel is halted in an immature stage prior to the initiation of spermatogonial proliferation under fresh water conditions, and a single injection of human chorionic gonadotropin (hCG) can induce spermatogenesis in this animal within 18 days (Miura et al. 1991a). Moreover, spermatogenesis can also be induced in vitro by hCG or 11-ketotestosterone (11-KT), the major androgens in teleosts, using an organ culture system (Miura et al. 1991b). We have previously elucidated a series of molecular mechanisms that underlie the action of various hormones in eel spermatogenesis using the organ culture method (Miura and Miura 2001), and recently we reported that a major progestin in teleost fish, 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) can induce the initiation of meiosis (Miura et al. 2006). This suggests that DHP plays key roles in early spermatogenesis.

To clarify the mode of action of DHP during spermatogenesis, we have previously carried out cDNA subtraction experiments using testicular fragments cultured with or without this progestin. Several cDNA clones that are regulated by DHP were thereby identified (Ozaki et al. 2006a, b; Miura et al. 2009), one of which showed a high homology to cysteine dioxygenase 1 (CDO1) in other animals. CDO is one of the enzymes that catalyze the conversion of L-cysteine to cysteine sulfinate (cysteine sulfinic acid). A subsequent reaction is catalyzed by cysteine sulfinate decarboxylase (CSD), which converts

cysteine sulfinate to hypotaurine. These reactions are essential for the biosynthesis of taurine. Our previous results suggested that DHP regulates taurine synthesis in eel testis. In the reproductive system in male vertebrates, taurine has been detected in Leydig cells, vascular endothelial cells, other interstitial cells of the testis, and epithelial cells of the efferent ducts in the rat (Lobo et al. 2000). Taurine may also act as an antioxidant in preventing rabbit sperm lipid peroxidation (Alvarez and Storey 1983), and as a sperm motility factor in the golden hamster (Boatman et al. 1990). These data implicate taurine also in fish spermatogenesis. In this regard, we have reported from our laboratory that progestin is an essential factor for the initiation of meiosis in eel (Miura et al. 2006), and our results indicated that progestin promotes taurine synthesis in the eel testis. We were therefore prompted to investigate whether taurine affects meiosis.

In our present study, we show that DHP regulates taurine synthesis in the eel testis by promoting the expression of CDO mRNA, and thus plays an important role in eel spermatogenesis.

Materials and methods

Animals and treatments

Cultivated male Japanese eel (*Anguilla japonica*) were purchased from a commercial eel supplier and were maintained in a 500-l circulating freshwater tank at 23°C prior to use. The experiments were conducted in accordance with the institutional animal ethics guidelines of Ehime University (Protocol Number 128, Permit Number H19-001).

Testicular organ culture

Testicular organ cultures were established as described previously (Miura et al. 1991a, b) with minor modifications. Briefly, freshly removed testes from eels were cut into $1 \times 1 \times 0.5$ mm^3 pieces and placed on floats of 1.5% agarose covered with a nitrocellulose membrane in 24-well plastic tissue culture dishes. The basal medium consisted of Leibovitz L-15 medium (Invitrogen, Carlsbad, CA) supplemented with 1.7 mM proline, 0.1 mM aspartic acid, 0.1 mM glutamic acid, 0.5% BSA, 1 mg/l bovine insulin, and 10 mM HEPES, adjusted to pH 7.4. Culture was done at ratio of two testicular fragments per 1.75 ml of L-15 medium.

cDNA subtraction

cDNA subtraction was performed as described previously (Ozaki et al. 2006a, b). Briefly, the testicular fragments were cultured for 6 days without or with DHP at a concentration of 100 ng/ml. Total RNA was extracted from the cultured testicular fragments using Sepasol RNA I Super (Nacalai Tesque, Kyoto, Japan). Poly (A) RNA was subsequently isolated from the total RNA preparations with Oligotex-dT-30 (Takara, Japan). cDNA subtraction was subsequently carried out using a Clontech PCR-Select cDNA subtraction kit (Clontech, Mountain View, CA, USA). The cDNA fragments encoding only differentially expressed genes were amplified using suppression PCR. Twenty-five cDNA fragments were identified from this screen, subcloned into a pGEM-T Easy Vector (Promega, Madison, WI) and sequenced using a Genetic Analyzer 3130 (Applied Biosystems, Tokyo, Japan). Among the cohort of fragments isolated, a single cDNA showed high homology to other vertebrate cysteine dioxygenases and the full-length cDNA was therefore obtained as described below.

Full cloning of CDO and CSD

cDNAs were reverse transcribed from the RNA preparations of Japanese eel livers using SuperScript II (Invitrogen). The reactions contained 0.5 μg of oligo$(dT)_{18}$ and 5 μg of total RNA in a 20-μl final volume. PCR was carried out using the degenerate primers listed in Table 1 (CSD-F1 and CSD-R1). Various cycle parameters were used until a PCR amplification product of the expected size was obtained (around 590 base pairs), based on the putative position of the primers within the CSD sequence. The amplified cDNA fragment was eventually obtained using the following cycle parameters: 94°C, 30 s; 60°C, 30 s; 72°C, 30 s for a total of 30 cycles.

To obtain the 5′- and 3′-ends of Japanese eel CDO and CSD, SMART cDNAs were generated from liver RNA using a SMARTer^{TM} RACE cDNA Amplification Kit (Clontech). 5′- and 3′-RACE was then performed in accordance with the manufacturer's instructions using the primers listed in Table 1. The CDO-RACE-R1/CDO-RACE-R2 and CSD-RACE-R1/CSD-RACE-R2 primers were used for 5′-RACE-PCR with the adaptor primers UPM and NUP included in the kit for first and secondary (nested) reactions, respectively. The CDO-RACE-F1/CDO-RACE-F2 and CSD-RACE-F1/CSD-RACE-F2 primers were used for the 3′-RACE-PCR. The obtained clones were sequenced using a Genetic Analyzer 3130 (Applied Biosystems).

Analysis of CDO and CSD expression

RT-PCR was performed to observe the effects of DHP and other sex steroids on CDO and CSD gene expression. After 3 days of culture, total RNA was isolated from Japanese eel testis cultured with DHP (10 ng/ml), 11-KT (10 ng/ml) and 17β-estradiol (1 ng/ml), and then treated with DNase I (Thermo Scientific, Yokohama, Japan). cDNA was

Table 1 Sequences of the primers used in the degenerate PCR, RACE and RT-PCR experiments

Primer	Sequence
CSD-F1	5′-AA(CT)(AC)CCA(AG)CC(AT)(AG)TACAC(AC)TACG-3′
CSD-R1	5′-AG(ACG)A(GT)(CT)TTGTG(AG)GG(AG)TTCA-3′
CDO-RACE-R1	5′-GCTGACGGCACTTTCCGTGTGGCTAA-3′
CDO-RACE-R2	5′-AGGCCCAGGGAGTCGTTTATGT-3′
CDO-RACE-F1	5′-GAACCTGGTGGACGAGGGGAATGGAA-3′
CDO-RACE-F2	5′-ATGATCCTCTGCTGGGGTGAAG-3′
CSD-RACE-R1	5′-ACGCTGCATCCACATGCATCCACA-3′
CSD-RACE-R2	5′-CTGTCCAGTGGGTCGAAGGAG-3′
CSD-RACE-F1	5′-CTTCTACCCCGGTGGCTCCGTCTCC-3′
CSD-RACE-F2	5′-GCCACTATTCCATCCGCAAAG-3′
CDO-RT-F1	5′-ATGATCCTCTGCTGGGGTGAAG-3′
CDO-RT-R1	5′-AGGCCCAGGGAGTCGTTTATGT-3′
CSD-RT-F1	5′-GCCACTATTCCATCCGCAAAG-3′
CSD-RT-R1	5′-CTGTCCAGTGGGTCGAAGGAG-3′
EF1α-RT-F1	5′-AGGCCCTTGACTCCATCCTG-3′
EF1α-RT-R1	5′-AGGCCCTTGACTCCATCCTG-3′

synthesized using SuperScript II and the primers used for RT-PCR are listed in Table 1. EF1α (GenBank accession number: AB593812) transcripts were used as the internal standard. For CDO amplification, the PCR cycling parameters were as follows: 28 cycles of 94°C for 30 s, 59.5°C for 30 s, and 72°C for 60 s. For CSD amplification, the PCR cycling parameters were as follows: 35 cycles of 94°C for 30 s, 57.5°C for 30 s, and 72°C for 60 s. The PCR products were electrophoresed on a 1.5% agarose gel, and stained with ethidium bromide.

Immunohistochemistry

Eel testes were sampled and fixed in Davidson's fixative, embedded in paraffin wax, and cut into 5-μm sections. Sections were subsequently deparaffinized in xylene and hydrated in a graded ethanol series. Both anti-rat CDO (Abcam, Tokyo, Japan) and anti-human CSD (Abnova, Taipei City, Taiwan) were used at a dilution of 1:10,000. Immunohistochemical analysis was performed using a Histofine SAB-AP (R) kit (Nichirei Biosciences, Tokyo, Japan). Negative controls were performed by omitting the primary antibody which yielded no reaction product (data not shown). The specificities of both antibodies were confirmed by western blotting.

Measurement of taurine in testis

After 6 days of culture, testicular fragments were collected, rinsed twice with eel Ringer's solution (150 mM NaCl, 3 mM KCl, $MgCl_2$, 5 mM $CaCl_2$, 10 mM HEPES, pH 7.4), and homogenized in 10 volumes of distilled H_2O with an ultrasonic homogenizer. Homogenates were then centrifuged at 1,000×*g*, and the supernatants were collected into a clean test tube, mixed with an equivalent volume of 10% trichloroacetic acid, and centrifuged at 10,000×*g* for 5 min at 4°C. Supernatants were collected into a clean test tube. A half volume of hexane was added to remove lipids via mixing and centrifugation at 10,000×*g* for 5 min at 4°C. The aqueous layer was filtered through a 0.22-μm syringe RC-membrane filter (Sartorius Stedim Biotech, Goettingen, Germany), and the taurine content was assayed by reverse-phase high-performance liquid chromatography (HPLC) (Hitachi, Tokyo, Japan) using an OPA reagent set (Wako, Osaka, Japan). Amino acid standard solution type B and type AN-II (Wako) were used as standards.

Effects of taurine on spermatogenesis in vitro

Testicular fragments were cultured for 6 days with taurine (0.08, 0.8, 8, or 80 μM; Wako), or with a combination of taurine (8 μM) and *β*-alanine (80 μM), a known competitive inhibitor of taurine uptake (WAKO). Prior to experiments, we confirmed the active uptake of taurine into testis in this culture system. Taurine levels in testicular fragments cultured with taurine (0, 0.8 or 80 μM) were 174.3 ± 4.57, 230.9 ± 4.08, 532.1 ± 35.03 nmol/100 mg testis, respectively, after 6 days of culture. To investigate the effects of taurine on 11-ketotestosterone (11-KT), eel testicular fragments were cultured with 11-KT (10 ng/ml) and taurine (0, 0.08, 0.8, 8, or 80 μM), or with 11-KT (10 ng/ml), taurine (0 or 8 μM) and *β*-alanine (0 or 80 μM). To investigate the effects of taurine on DHP, the testicular fragments were cultured with DHP (1 ng/ml) and taurine (0, 0.08, 0.8, 8, or 80 μM), or with DHP (1 ng/ml),

taurine (0 or 8 μM) and β-alanine (0 or 80 μM). Testicular fragments cultured without taurine or any sex steroids were used as controls. After 6 days of culture, germ cells were labeled with 5-bromo-2-deoxyuridine (BrdU) in accordance with the manufacturer's instructions (Amersham Bioscience, Uppsala, Sweden) to analyze the effect of taurine on spermatogenesis. In the experiment, testicular fragments were incubated with BrdU (1 μl/well) for 18 h, fixed in Bouin's solution, embedded in paraffin, and cut at 5 μm thickness. The sections were then stained immunohistochemically using an antibody against BrdU and counterstained with Delafield's hematoxylin. The number of immunolabeled germ cells was counted and expressed as a percentage of the total number of germ cells. Results are expressed as mean ± SEM of five replicates. Data analysis was carried out using one-way ANOVA followed by Bonferroni test. Significance was accepted at $P < 0.05$.

Testicular fragments were also cultured for 15 days with taurine (0 or 8 μM) and as a control with 11-KT (10 ng/ml), as this was identified in our previous report to cause complete spermatogenesis (Miura and Miura 2001). The cultured testicular fragments were subsequently fixed in Bouin's solution, embedded in paraffin, and cut. The sections were then stained with Delafield's hematoxylin as described above.

Western blot analysis

To investigate the effects of taurine on meiosis, we analyzed the expression of Spo11, a meiosis-specific marker, using a modified western blot method as previously described (Miura et al. 2006). Briefly, cultured testicular fragments were homogenized in 20 mM Tris–HCl (pH 8.0) containing 1 mM EDTA, 1 mM PMSF and centrifuged at 10,000×*g* for 10 min at 4°C. The supernatant was mixed with an equal volume of sample buffer [0.125 M Tris–HCl at 4% (wt/vol), SDS at 20% (vol/vol), glycerol at 0.05% (wt/vol), and bromophenol blue] with 10% 2-mercaptoethanol. All samples were boiled for 10 min after mixing with sample buffer. The protein concentration was determined using a protein assay rapid kit (Wako).

For western blot analysis, proteins (10 μg) were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The membrane was immersed overnight at 4°C in a solution containing anti-eSpo11 at a dilution of 1:2,000 in Can Get Signal solution 1 (Toyobo, Osaka, Japan). After washing, the membrane was incubated with alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG (Vector Laboratories, Marion, IA, USA) diluted 1:3,000 in Can Get Signal solution 2 (Toyobo) for 1 h. After washing, AP activity was visualized using CDP-Star Detection Reagent (GE Healthcare, Tokyo, Japan) and analyzed by using a LAS-4000 device (GE Healthcare).

Results

Cloning of CDO and CSD cDNAs from the Japanese eel (*Anguilla japonica*)

The CDO cDNA isolated from the Japanese eel was found to be 740-bp long, with an ORF encoding a protein of 201 amino acids (DDBJ/EMBL/GenBank accession number AB638837) and showed high similarity to the CDO sequences of other organisms. Moreover, in the common carp (*Cyprinus carpio*), two isolated CDOs (CDO1 and CDO2) have been reported (Honjoh et al. 2010). The similarity between the predicted proteins of eel CDO to those of CDO1 and CDO2 is 86 and 83%, respectively. In addition, the five amino acid residues at the C terminus of eel CDO (Pro–Phe–Glu–Thr–Thr) and carp CDO1 (Pro–Tyr–Glu–Ser–Thr) are similar, whereas carp CDO2 is divergent (Ala–Tyr–Ala–Gly–Ser). The cDNA, therefore, obtained in this study was thus shown to be Japanese eel CDO1.

The isolated eel CSD cDNA fragment was 2,261-bp with a predicted amino acid sequence of 520 residues (accession number AB638838). This product showed an approximately 75% similarity to other fish CSDs and 65% similarity to mammalian CSDs.

Effects of sex steroids on CDO1 and CSD expression in the eel

To see the effects of sex steroids (DHP, 11-KT and 17β-estradiol) on CDO1 and CSD expression, RT-PCR were performed using specific primers for eelCDO1 and eelCSD. As a result, we observed that the CDO1 gene alone is highly expressed in eel testicular fragments cultured with DHP, whilst the CSD gene was found to be expressed in cultured eel testis but no remarkable differences in its expression levels were evident among the groups (Fig. 1).

Localization of CDO and CSD in eel testis

To determine the distribution of CDO1 and CSD in an immature eel testis, we performed immunohistochemistry using anti-CDO and anti-CSD antibodies (Fig. 2). Both CDO1 and CSD were found to be expressed in the Sertoli cells surrounding spermatogonia whereas no expression of either of these proteins was found in spermatogonium or interstitial cells.

Effects of DHP on taurine synthesis in the eel testis

HPLC analysis revealed that DHP treatment at all concentrations led to an increased taurine level in cultured eel testes. Notably, a 10 ng/ml DHP treatment led to a significantly increased taurine level in this tissue

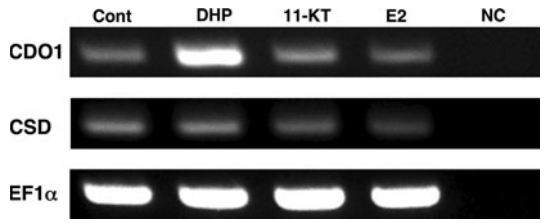

Fig. 1 Results of RT-PCR analysis specific primers for eelCDO1 and eelCSD. Testicular fragments were cultured with 10 ng/ml of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP), 10 ng/ml of 11-ketotestosterone (11-KT) or 1 ng/ml of estradiol-17β (E2). EF1α transcripts were used as an internal standard. The amplification reaction in the negative control (NC) lane was performed in the absence of reverse transcriptase

(257.9 ± 8.0 nmol/100 mg testis) compared with a control (206.2 ± 9.6 nmol/100 mg testis) (Fig. 3).

Effects of taurine on eel spermatogenesis

We next investigated the effects of taurine during early eel spermatogenesis using an in vitro organ culture system. Testicular fragments from the eel were cultured with increasing concentrations of taurine (0.08, 0.8, 8 or 80 μM) for 6 days. DNA synthesis in spermatogonia was then investigated by exposing these tissues to BrdU. The addition of 0.8, 8 or 80 μM taurine into the culture medium induced significant DNA synthesis in a dose-dependent manner (Fig. 4a). β-alanine administration (80 μM) was found to significantly inhibit the DNA synthesis induced by taurine (Fig. 4b). Taurine was further found to promote DNA synthesis induced by 11-KT or DHP (Figs. 5a, 6a), effects which were inhibited by β-alanine (Figs. 5b, 6b).

To evaluate the effects of taurine on meiosis, western blot analysis was performed using an anti-eel Spo11 antibody. Faint bands were detected in the initial control, control and low dose taurine (0.08, 0.8 and 8 μM) cultured groups. However, stronger signals were detected in testes cultured with an 80-μM dose of taurine. Specific bands were also detected in DHP-treated testicular fragments with a molecular mass of 37 kDa (Fig. 7). In long-term cultures (15 days), only spermatogonial cells were observed in taurine-treated group at any concentrations and post-mitotic cells such as spermatocytes were not observed. On the other hand, spermatogonia and spermatocytes were observed in the 11-KT-treated group. In the control group, only spermatogonia were observed throughout the entire experimental period (Fig. 8).

Discussion

To investigate the actions of DHP during spermatogenesis in the Japanese eel, we carried out a cDNA subtraction screen using testicular fragments cultured with or without DHP. The results showed that one of the cDNA clones up-regulated by DHP stimulation encoded CDO1. The deduced amino acid sequence of this eel CDO1 product showed high similarity with this enzyme in other organisms.

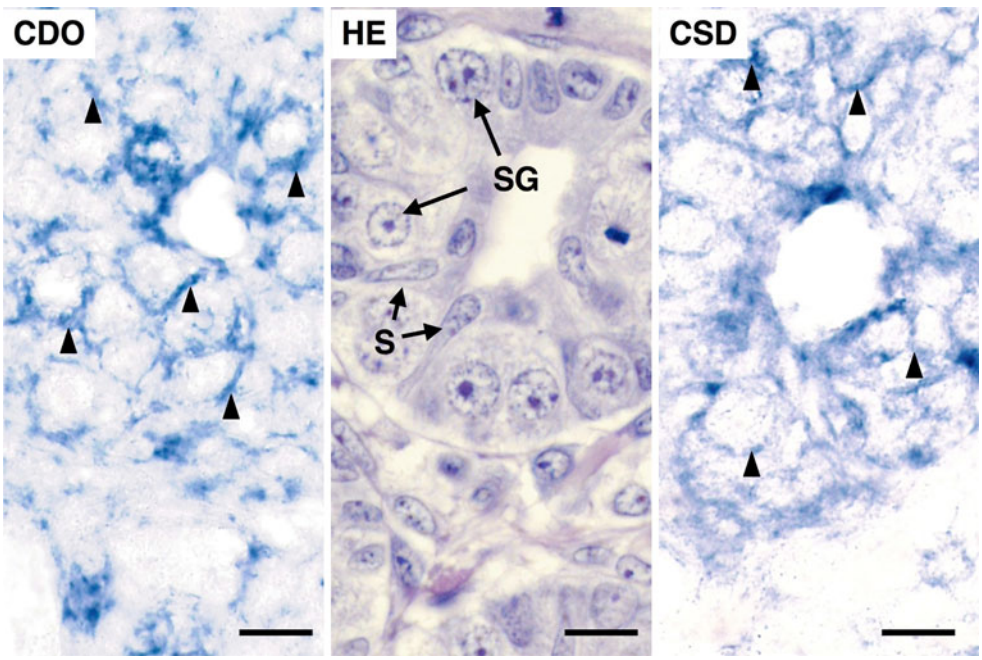

Fig. 2 Localization of CDO and CSD in eel testis assessed by immunohistochemistry. *CDO* testis immunostained with anti-CDO antibodies, *HE* testis section stained with hematoxylin and eosin, *CSD* testis immunostained with anti-CSD antibodies, *SG* spermatogonia, *S* Sertoli cell. *Arrowheads* indicate areas of positive immunoreactivity. *Bar* 10 μm

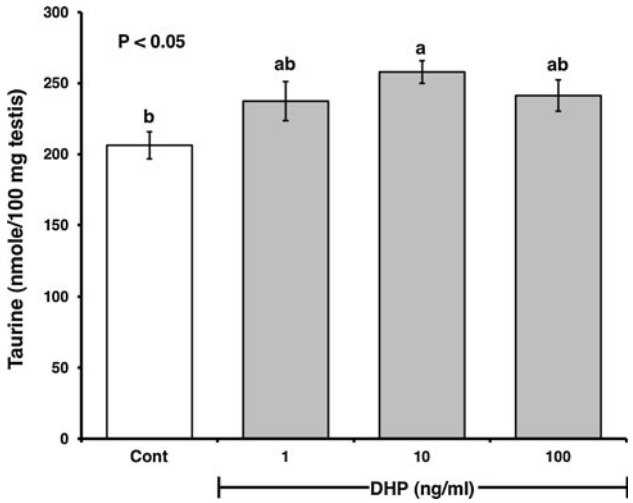

Fig. 3 Taurine levels in cultured eel testicular fragments. Values are the mean ± SEM ($n = 6$). *Different letters* indicate significant differences ($P < 0.05$)

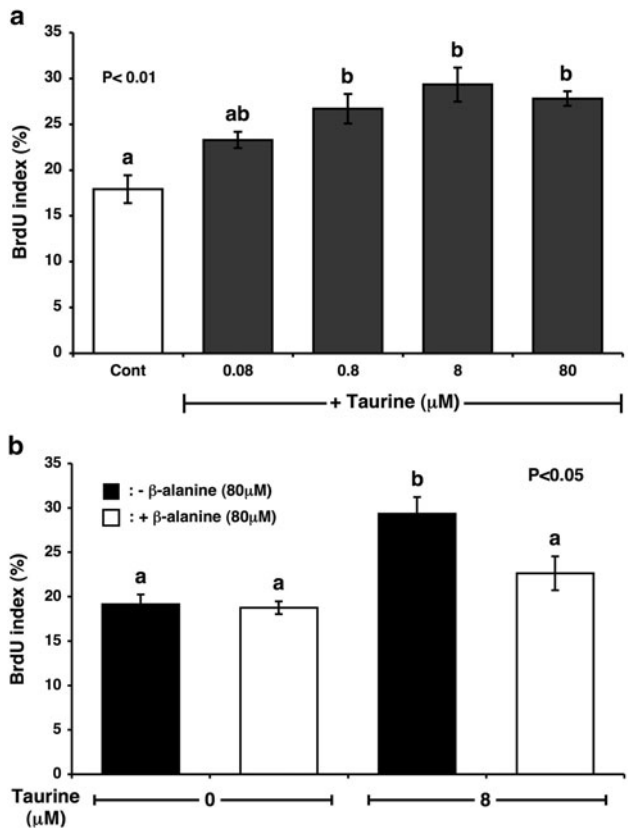

Fig. 4 BrdU labeling index of germ cells in eel testicular fragments cultured with various concentrations of taurine (**a**), or cultured with/without taurine and with/without β-alanine (**b**). Values are the mean ± SEM ($n = 6$). *Different letters* indicate significant differences [$P < 0.01$ (**a**) or $P < 0.05$ (**b**)]

CDO catalyzes the conversion of L-cysteine to cysteine sulfinate (cysteine sulfinic acid), which is an essential step in taurine biosynthesis. Cysteine sulfinate is subsequently

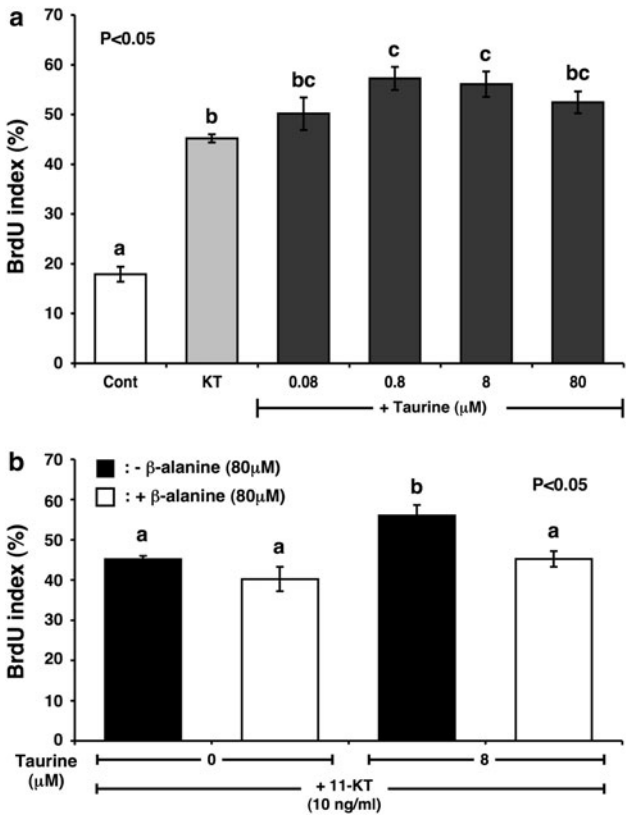

Fig. 5 BrdU labeling index of germ cells in eel testicular fragments cultured with 11-KT and various concentrations of taurine (**a**), or with 11-KT in combination with/without taurine and with/without β-alanine (**b**). Values are the mean ± SEM ($n = 6$). *Different letters* indicate significant differences ($P < 0.05$)

converted into hypotaurine by cysteine sulfinate decarboxylase (CSD). Hypotaurine is believed to be auto-oxidized to taurine because no gene has yet been identified in any organism that catalyzes this process. The fact that DHP up-regulates CDO1 expression in the testis suggests that taurine may be synthesized in this organ and that DHP may regulate taurine synthesis. In our present study, to determine whether DHP regulates taurine synthesis in eel testis, we first tried to isolate the cDNA clones corresponding to genes encoding CSD in the eel. The deduced amino acid sequence of the eel CSD clone that emerged from this screen showed close similarity to that of the CSD sequences reported in other organisms, including a pyridoxal phosphate binding motif (NPHK) (Kaisakia et al. 1995) which is conserved in the eel CSD protein. It was further confirmed by RT-PCR that both CDO1 and CSD transcripts are expressed in eel testis. Moreover, the results of our RT-PCR analysis revealed that DHP specifically up-regulates CDO1 mRNA expression. There has been no information reported to date indicating that progestin regulates CDO1 mRNA in testis or in other tissues. To our knowledge therefore, our current report is the first to do so.

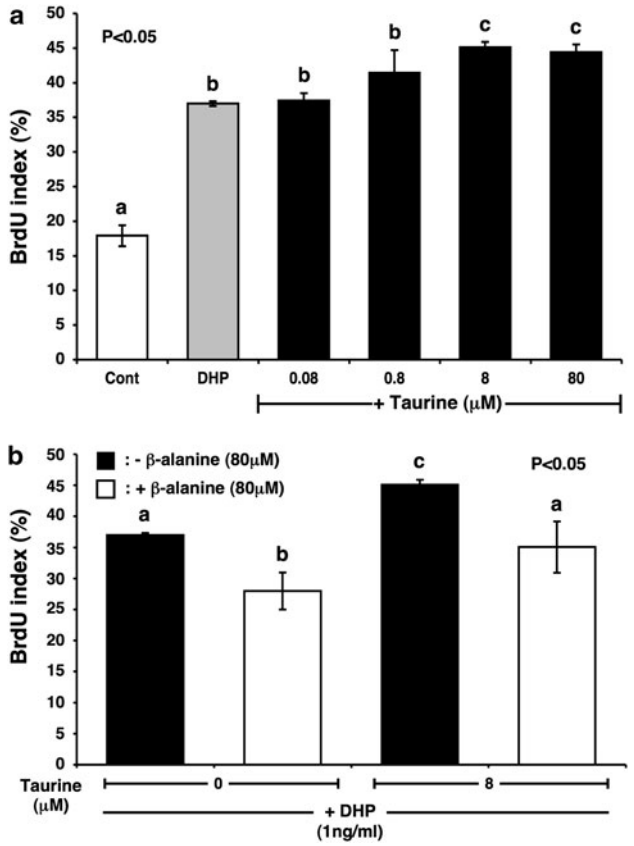

Fig. 6 BrdU labeling index of germ cells in eel testicular fragments cultured with DHP and various concentrations of taurine (**a**), or with DHP in combination with/without taurine and with/without *β*-alanine (**b**). Values are the mean ± SEM ($n = 6$). *Different letters* indicate significant differences ($P < 0.05$)

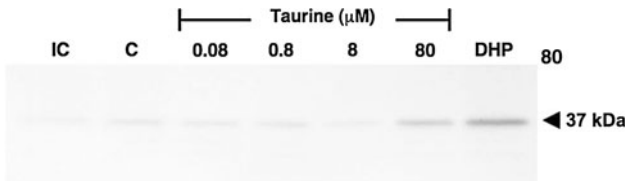

Fig. 7 Expression of the meiosis-specific marker protein Spo11 in cultured eel testis. Testicular fragments were cultured with 0.08, 0.8, 8, or 80 μM of taurine. An initial control sample taken before culture and a control cultured without taurine are indicated in the initial control (*IC*) and control (*C*) lanes, respectively. As a positive control for the expression of Spo11, testis tissue fragments were cultured with 10 ng/ml of DHP for 6 days. Western blots were performed five times in independent animals yielding similar results

By RT-PCR analysis, we further showed that CSD mRNA is expressed in cultured eel testis. However, no significant differences were found in the CSD mRNA expression levels between DHP-treated, 11-KT-treated, E2-treated and untreated testes. This means that sex steroids we examined (DHP, 11-KT, E2) do not affect CSD mRNA expression in eel testis. Hence, our present data indicate that DHP up-regulates CDO1 transcription only.

We additionally found that both CDO1 and CSD are expressed in Sertoli cells in the eel. 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 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 brain (Tappaz et al. 1999), kidney (Park et al. 2002), and testis (Li et al. 2006). In our present study, we have demonstrated an in vitro regulatory effect of DHP in the eel testis that results in increased taurine concentrations. The formulation of L-15 culture medium indicates that no taurine or hypotaurine is present, and this was confirmed by HPLC analysis (data not shown). Hence, the increased taurine in the testis is newly synthesized during in vitro organ culture and DHP promotes taurine synthesis in this organ via the up-regulation of CDO mRNA.

It has been reported previously that an increase in CDO activity increases taurine production (Bagley and Stipanuk 1995) and suggested that CDO is rate-limiting for taurine synthesis (Joel and James 1988). This lends support to the hypothesis that DHP promotes taurine synthesis in the eel testis by up-regulating CDO mRNA expression. However, no other evidence has been reported to date suggesting that the up-regulation of CDO mRNA expression leads to taurine synthesis. To validate this finding, therefore, it will be necessary to investigate the relationship between the CDO mRNA level and the activity levels of this enzyme in a future study.

Our present data further suggest that taurine has a key role in eel spermatogenesis. Taurine has been shown to have various functions such as bile formation in the liver (Vessey 1978), osmoregulation (Lasserre and Gilles 1971), the stabilization of membranes (Huxtable and Bressler 1973), and calcium binding and transport regulation (Lazarewicz et al. 1985; Lombardini 1985). Taurine also acts as an antioxidant and protects against the toxicity of various substances in vivo (Green et al. 1991; Gürer et al. 2001; Balkan et al. 2002; Sinha et al. 2008). In the reproductive system of male rats, taurine has been detected in Leydig cells, vascular endothelial cells, other interstitial cells of the testis, and epithelial cells of the efferent ducts (Lobo et al. 2000). Taurine can also stimulate testosterone secretion in the rat testis in vivo and in vitro (Yang et al. 2010). In teleost fish, there is little information currently available regarding the role of taurine in the testis. However, taurine has been detected in the seminal fluids of the carp and trout (Billard and Menezo 1984). Taurine may also act as an antioxidant that prevents sperm lipid peroxidation in rabbit (Alvarez and Storey 1983), and as a sperm motility factor in the golden hamster (Boatman et al. 1990). We thus examined in our current study the direct

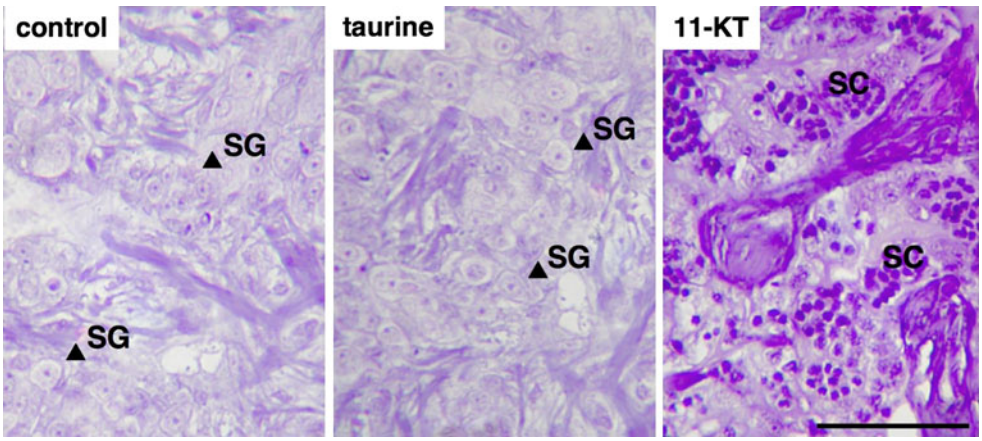

Fig. 8 Histology of a 15-day cultured eel testis from a control, taurine-treated and 11-KT-treated group. *SG* spermatogonia, *SC* spermatocyte. *Scale bar* 50 μm

effects of taurine on eel spermatogenesis using an organ culture system. The results of a short-term (6 days) culture showed that DNA replication in spermatogonia is induced by taurine but suppressed by β-alanine, a known competitor of taurine transport. Moreover, taurine was found to enhance the induction of germ cell proliferation by 11-KT or DHP, effects which were also suppressed by β-alanine.

We have reported previously that 11-KT and DHP are essential factors for the initiation of meiosis (Miura and Miura 2003; Miura et al. 2006) and we here show that DHP promotes taurine synthesis in the eel testis via the up-regulation of CDO expression. Our current data suggested that taurine may be involved in meiosis and we thus examined the effects of taurine on the expression of Spo11, a meiosis-specific marker. Spo11 has been identified in several vertebrates previously and its function has been found to be widely conserved in eukaryotes (Romanienko and Camerini-Otero 1999; Keeney 2001). It is also known that Spo11 protein initiates meiotic recombination by generating DNA double-strand breaks, and was suggested by our previous data that the entry of spermatogonia into meiosis is a critical step for the progression of spermatogenesis (Miura et al. 1999). Western blot analysis in our current experiments revealed that taurine promotes Spo11 expression implicating taurine as one of the factors involved in the initiation of meiosis. However, the results of long-term (15 days) organ culture showed that taurine cannot induce complete spermatogenesis. Consistent with this finding, we reported previously that trypsin is also up-regulated in eel testis by DHP and that it induces the expression of Spo11 in germ cells, but not all aspects of spermatogenesis (Miura et al. 2009). These results collectively suggest that meiosis is controlled by the conglomeration of various factors including taurine and trypsin.

We conclude from our current data that progestin promotes taurine synthesis in the eel testis via up-regulation of CDO mRNA expression and that taurine is one of factors that mediates the control of spermatogenesis.

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