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Prostaglandin E synthase is regulated in postnatal mouse testis

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1. ABSTRACT

Prostaglandins have important roles in the male reproductive system. In this study, we report on the distribution and regulation of cPGES during postnatal development of mouse testis. The expression of cPGES was weak in testis 5 days after birth and increased through the 10th and 15th day. From the 20th day onward, the cPGES expression in testis reached the level of adult mice. cPGES was expressed at a constantly low level in Sertoli cells in the testis from infant to adult stages. With the occurrence of meiosis during puberty, a high level of cPGES was detected in the spermatocytes and round spermatids, which was then maintained throughout the adulthood. In addition, cPGES was found highly expressed in the epididymis, seminal vesicles and vas deferens This suggests that cPGES in Sertoli cells in infant to juvenile mouse testis contributes to a basic PGE2 synthesis in seminiferous tubules. However, the high level of cPGES in spermatocytes and spermatids may maintain a high amount of PGE2 in seminiferous tubules, which may be tightly coupled with the spermatogenic cycle.

2. INTRODUCTION

Prostaglandin E2 (PGE2) is the most common prostaglandin (PG) hormone in mammals. It is found in a variety of tissues and cells and has a broad range of bioactivity. PGE2 regulates key responses in human reproductive, renal, gastrointestinal, cardiovascular, neuroendocrine, and immune systems. In addition, PGE2 plays crucial roles in various pathologic reactions, such as fever, pain hypersensitivity, inflammation, vascular hypertension, and tumorigenesis (1, 2)

PGE2 is produced by three enzymatic reactions. Phospholipase A2 (PLA2) enzymes hydrolyze membrane glycerophospholipids into arachidonic acid (AA). AA is metabolized into the unstable intermediate prostanoid PGH2 by cyclooxygenase (COX) enzymes and then to PGE2 by terminal prostaglandin E synthases (PGES) specifically (3, 4). Each of these three reactions

can be rate-limiting and involve multiple enzymes that act in different phases of cell activation and exhibit distinct functional coupling (4).

Previous studies suggest the presence of four distinct PGES. Among these, two forms are membrane-associated PGES, termed mPGES-1 (5, 6) and mPGES-2 (7), and the other two are cytosolic forms of PGES, named cPGES (8), and mu-class glutathione-S-transferases (GSTs) (9). mPGES-1 is an inducible enzyme localized to the nuclear and microsomal membranes, preferentially coupling with the inducible COX-2. It is expressed in a low basal level in normal tissues and is markedly up-regulated in response to various proinflammatory stimuli such as TNFα and IL-1β in brain, lung, spleen, stomach, kidney, and seminal vesicles to promote delayed PGE2 generation (10, 11). mPGES-2 was originally purified from microsome of bovine heart cells. It is expressed constitutively in several tissues in which mPGES-1 expression is relatively low. It can be coupled with both the constitutive enzyme COX-1 and the inducible enzyme COX-2. The mu-class GSTs are mainly localized to the central nervous system and also convert PGH2 to PGE2 specifically in the presence of glutathione (9).

cPGES is expressed constitutively in the cytosol of a variety of cells and tissues. Expression of cPGES was not affected by the proinflammatory stimuli with an exception in rat brain in which lipopolysaccharide treatment increases its amount several-fold (8). cPGES converts the constitutive enzyme COX-1-, but not the inducible enzyme COX-2-derived PGH2 to PGE2, particularly during the immediate PGE2 biosynthetic response resulting from Ca^{2+}-evoked stimuli, during which high concentrations of AA are released in a short period, indicating that the physiological role of cPGES may be to produce the PGE2 required for the maintenance of homeostasis (8, 11).

Both the male reproductive tract and semen fluid are reported to contain large amounts of prostaglandins which are mainly composed of PGE2 (12-16). In the male reproductive system, prostaglandins have important roles in various processes. Didolkar et al reported that PGE2 caused a significant increase in rat testicular weight, RNA content, hyaluronidase activity, and number of spermatids. It is suggested that PGE2 may be involved in the later stages of spermatogenesis, i.e. conversion of spermatocytes to spermatids (17). Aitken et al reported that PGE2 treatment increases human sperm motility and penetrating ability (18). High concentrations of PGE in seminal plasma have been suggested to be involved in sperm transport through the female reproductive tract and subsequent fertilization (19). In the acrosome reaction, PGE1 and PGE2 promote calcium influx through pertussis toxin-insensitive Ca^{2+} channel. Extracellular Ca^{2+} influx contributes to the acrosome membrane and sperm plasma membrane fusion, and thus, sperm acrosome reaction is induced (20). Several reports have appeared linking low levels of PGE in human semen with unexplained infertility (17). Treatment with anti-PGE2 serum significantly decreased rabbit semen fertilization rate through artificial insemination, but sperm motility was not affected (21). By the contrast, the addition of exogenous PGE to semen has been shown to improve fertility in both equine (22) and human studies (23). It seems that functional requisites of sperm production, maturation, transport, acrosome reaction, and fertilization depend upon optimal seminal PGE concentrations. However, a few experiments conducted on the influence of PGEs on human sperm function have provided conflicting results (17).

There are still controversies about the source of seminal PGE2. Several reports determine the site of prostaglandin synthesis through the examination of the content of prostaglandins in the male reproductive tract. To date, a large amount of PGE2 is shown by several reports to be produced in the seminal vesicle (12-14). However, it is not certain whether PGE2 is produced in the testis (12, 15, 24-28). The wide range of PG concentrations recorded in the semen as well as technical problems of the semen storage and the accurate measurement of PG levels render the results incompatible (17). To identify the location of prostaglandin biosynthesis, it might be an effective alternative to explore the distribution of prostaglandin synthases.

Testis shows a high activity of prostaglandin synthesis, comparable to the epididymis and seminal vesicular gland (28). The enzyme for the synthesis of PGE2, including PLA2, COX, and mPGES, has been found in the testis (29, 30). However, little is known about the expression and regulation of cPGES in the male reproductive tract, except for that *cPGES* mRNA was found in the seminiferous tubules, Leydig cells and epididymis (31). To elucidate the biological roles of cPGES in the male reproductive system, we investigated cPGES protein distribution within the mouse reproductive tract and the alteration of its expression in the testis during the development after birth.

3. MATERIALS AND METHODS

3.1. Animals

Infant, juvenile, and adult mice of Kunming strain were purchased from the experimental animal centre of Shandong University, China. The mice were maintained in a standard animal facility. Permission to use animals in this study was granted by Shandong University Institutional Review Board.

3.2. Tissue preparation

Male mice were anaesthetized and sacrificed by cervical dislocation. The testes were removed and decapsulated. One testis was frozen in liquid N_2 for subsequent Western blot analysis. The contralateral testis was fixed overnight at 4 ^{o}C in 4% paraformaldehyde. After embedment in paraffin, the testes were sectioned (6 μm-thick) and mounted onto gelatin coated slides. For immunohistochemistry, each slide contained testis sections from infant, juvenile, and adult mice in order to subject the different tissues to the same treatment and thus make the

results more comparable. The testis, epididymis, vas deferens, and seminal vesicles of adult mice were isolated and collected for Western blot detection.

3.3. Isolation and culture of Sertoli cells

The Sertoli cells were isolated from infant (5 days old) or adult (90 days old) mouse testis by a method previously described (32) with minor modifications. Briefly, isolated seminiferous tubules were minced into small pieces and then incubated in PBS containing 0.1% collagenase (type 1) at 33 ^{o}C for 15 min with gentle shaking. After centrifugation at 600 g for 15 min, cell population was incubated again in PBS containing 0.25% trypsin at 33 ^{o}C for 10 min with occasional pipetting, followed by the termination of trypsin digestion by adding fetal bovine serum to 10% (v/v). The resulting cell suspension was filtered through 100 μm steel mesh to remove cell aggregates and tissue debris. Then cells were collected from the percolate by centrifugation and resuspended in Dulbecco Modified Eagle Medium (DMEM) containing 10% fetal bovine serum. The cell suspension was plated in a six-well plate and was incubated in a humidified atmosphere of 5% CO_2 in air at 33 ^{o}C to allow the Sertoli cell attachment, followed by a change of medium to remove suspending germ cells. The purity of the resulting Sertoli cells was determined by immunocytochemistry with anti-vimentin antibody (33) (Santa Cruz) and was routinely about 95%.

3.4. Isolation of spermatocytes and round spermatids

The spermatocytes and round spermatids were isolated from 90-day-old adult mice. The main procedures, such as isolation, digestion, and filtration, were as described above. The resulting cells were incubated in 90 mm plate for 12 h to allow somatic cell attachment. The suspending cells were enriched and resuspended in ice-cold 2.5% percoll/PBS solution containing 5 mmol/L lactate, 1 mmol/L pyruvate, and 0.1% BSA. Then the cells were separated by sedimentation through a 5–12% percoll gradient at 4°C for 1 h. Gradient fractions were examined by microscopy and distinguished according to the diameter of the cells. The fraction enriched for spermatocytes and round spermatids were pooled respectively and transferred onto gelatin coated slides. The purity of spermatocytes, round spermatids was determined by immunocytochemistry with anti-SCP-3 (synapsis complex protein-3) (34) antibody (Santa Cruz) and was routinely about 85%.

3.5. Western blot analysis

Samples to be analyzed by Western blotting from mice of different ages at 5, 10, 15, 20, 30 and 90 d were homogenized in ice-cold extraction buffer containing 20 mmol/L Hepes, 15 mmol/L $MgCl_2$, 50 mmol/L NaF, 1 mmol/L dithiothreitol, 3 μg/mL Leupeptin, 80 mmol/L β-glycerophosphate, and 50 mmol/L PMSF. The crude homogenates were centrifuged at 12,000 g for 20 min at 4 °C and the resultant supernatant contained the soluble protein fraction. Total protein concentration was determined using the DC protein assay kit (Bio-Rad) according to the manufacturer's instructions. For each sample, 40 μg protein was diluted with sample buffer (60 mmol/L Tris-Cl, pH 6.8, 200 mmol/L dithiothreitol, 2% SDS, 0.1% bromophenol blue, 25% glycerol) and heated to 95 °C for 10 min, followed by electrophoresis in a 10% polyacrylamide gel and transferred onto an Immuno-blot PVDF Membrane (Bio-Rad) using the Bio-Rad system according to the method described previously (35). The membranes were blocked with 10% non-fat dry milk in TBST (0.1 % Tween-20 in TBS) for 1 h at room temperature, then probed overnight at 4 ^{o}C with rabbit anti-mouse cPGES polyclonal antibody (Cayman Chemical, diluted 1:1,000) or anti-mouse β-actin monoclonal antibody (Santa Cruz, diluted 1:1,000) in TBST with constant shaking. The membranes were then washed in TBST for 6-10 min, and incubated with secondary antibody (goat anti-mouse IgG-AP or goat anti-rabbit IgG-AP, Santa Cruz, diluted 1:1,000) for 1 h at room temperature. Following several washes in TBST, the signal was visualized by incubation in NBT/BCIP (Roche, diluted 1:50 in 100 mmol/L Tris–Cl, pH 9.6, 50 mmol/L MgCl2, 100 mmol/L NaCl, 0.1% Tween-20) for 2-5 min. Band intensity was quantified by use of Quantity One software (Bio-Rad) and normalized to β-actin level.

3.6. Immunohistochemistry

The localization of cPGES protein in mouse testes was mapped using immunohistochemistry as described previously (35). The dewaxed and rehydrated testicular sections were incubated in 2% H_2O_2 diluted in methanol for 15 min to quench the endogenous peroxidase. After treatment with 10 μg/mL saponin (Sigma) for 30 min to retrieve the antigen, the sections were blocked with 1.5% normal sheep serum (Sigma) in TBS (pH 7.5) at room temperature for 2 h. Primary antibodies included anti-mouse cPGES polyclonal antibody (Cayman Chemical), anti-mouse vimentin monoclonal antibody (Santa Cruz), and anti-mouse SCP-3 monoclonal antibody (Santa Cruz), which were diluted 1:200 in TBS containing 1% goat serum and 0.1% Triton X-100. Tissue sections were exposed to diluted primary antibody overnight at 4 °C. Thereafter, the sections were incubated with biotinylated anti-rabbit or anti-mouse secondary antibody provided in avidin-biotin-peroxidase complex kit (Vector). Then the sections were incubated for 30 min with ABC reagent from the same kit. Slides were washed thoroughly in TBS after each step. Finally, sections were reacted with diaminobenzidine tetrahydrochloride (provided in the kit) until desired staining.

3.7. Immunofluorescent staining of isolated Sertoli cells and germ cells

Both cultured Sertoli cells and separated germ cells in smear were fixed with methanol at -20 °C for 5 min, permeabilized with 0.5% Triton X-100 for 5 min at 4 °C, and then blocked with 5% normal sheep serum (Sigma) in TBS (pH 7.5) at room temperature for 2 h. Cells were incubated with diluted (1:100) anti-mouse cPGES, anti-mouse vimentin, or anti-mouse SCP-3 primary antibodies, respectively, for 2 h at room temperature, followed by FITC conjugated anti-mouse IgG or Phalloidin-Texas Red conjugated anti-rabbit IgG (diluted 1:50) for 1 h at room temperature in a humidified chamber. After three 5-min washes in TBST, protein localization was determined by laser confocal microscopy.

3.8. Statistical analysis

All data are presented as means ±S.E. and all statistical analyses were performed with Student *t*-test

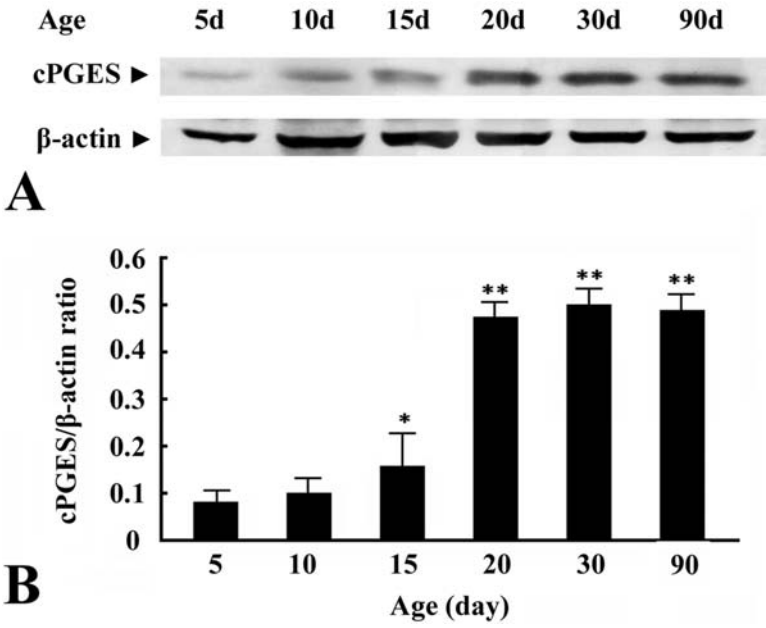

Figure 1. cPGES expression in mouse testes during postnatal development. (A) The testes isolated from different ages of mice were examined by Western blot. A representative immunoblot of cPGES and the protein loading control β-actin are shown. (B) Quantitative densitometry of cPGES and β-actin immunoblot bands. The density of each band was quantified by the use of Quantity One software. The relative expression level of cPGES was measured by cPGES /β-actin ratio. Results are the mean±S.E. from four independent experiments. *$p < 0.05$, **$p < 0.01$, versus 5-day-old mouse testis.

using SPSS 13.0 software. The level of significance was set at $p < 0.05$ and $p < 0.01$.

4. RESULTS

4.1. Expression level of cPGES in mouse testis during postnatal development

Western blot analysis was performed to examine the variability of cPGES expression in mouse testis during postnatal development. The level of cPGES was low in 5-day-old mouse testes and increased gradually though 10 to 15 d. In 20-day-old mouse testes, cPGES level increased by about 5- fold compared with that in the 5-day-old mouse testes and reached the peak. After that it remained stable through the adult stage (Figure 1). In sum, cPGES is expressed at a low level in the infant testis, dramatically increases during puberty, and thereafter maintains high level throughout the adulthood.

4.2. Distribution of cPGES in mouse seminiferous epithelium during postnatal development by immunohistochemistry

In order to characterize the cell types in which cPGES is expressed, immunohistochemistry was used to localize the cPGES in the testis during postnatal development. Anti-mouse vimentin and anti-mouse SCP-3 monoclonal antibodies were used to indicate the position of the Sertoli cells and spermatocytes in seminiferous tubules from different aged mice respectively (33, 34). In sections of seminiferous tubules from 5-day mice, the vimentin rather than SCP-3 signal was strong in almost all cells and the cPGES signal was concurrent with the vimentin signal, indicating that cPGES was expressed originally in the Sertoli cells in infant mouse testes when spermatogenic cells other than spermatogonia were not present (Figure 2). In sections of seminiferous tubules from 10-day mice, primary spermatocytes, which were first seen in a small amount of seminiferous tubules, were strongly marked by the SCP-3 antibody. Meanwhile, high levels of cPGES were detected in the cytoplasm of these cells. Sertoli cells were also stained by cPGES antibody, but the signal was weaker than that in the primary spermatocytes. During the juvenile period, spermatocytes, round spermatids, and elongated spermatids were gradually present in the seminiferous epithelium, and the Sertoli cells were mainly located at the base of seminiferous tubules, as shown on the 15- to 30-day-old mouse testis sections (Figure 2). cPGES was mainly expressed in the cytoplasm of spermatocytes and round spermatids. In adult testes, the expression pattern of cPGES was similar to 30-day-old testes. During the development from infant to adult, the proportion of the Sertoli cells in seminiferous epithelium gradually decreased, while the expression of cPGES in the Sertoli cells seemed stable. The immuno-signal found in the Leydig cells was as weak as in the Sertoli cells.

4.3. Immunofluorescent localization and expression level of cPGES in the isolated Sertoli cells and germ cells

To verify the immunohistochemistry results, double immunofluorescent staining of the isolated Sertoli cells and separated germ cells was performed. The primary culture of Sertoli cells, which were isolated from infant or adult mouse testes, was grown and adhered to glass

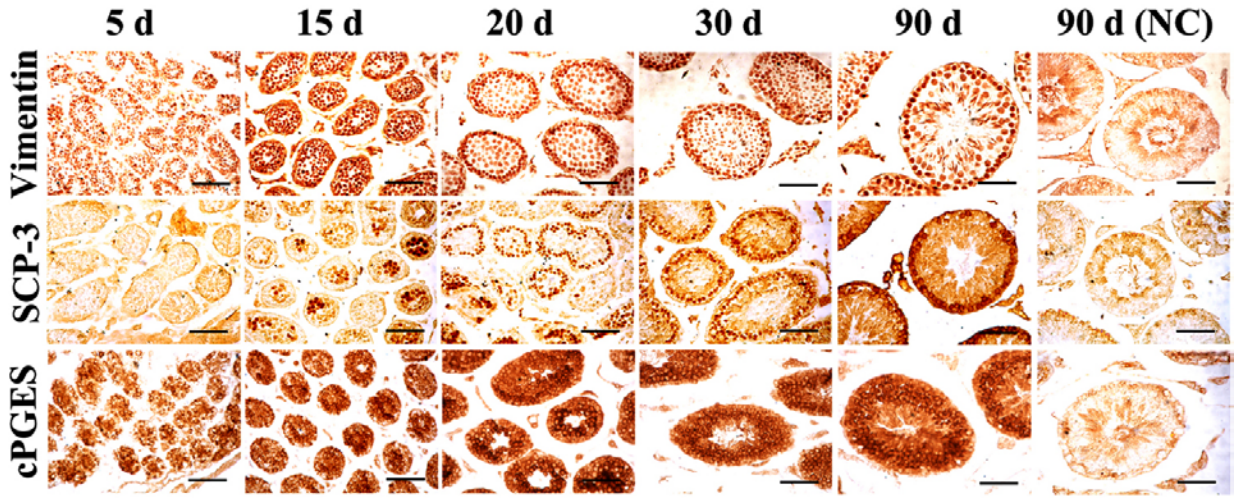

Figure 2. Immunohistochemistry demonstrating cPGES expression and localization in mouse seminiferous epithelium during postnatal development. Sections from mice ages 5, 15, 20, 30, and 90 d were mounted onto the same slide in order to subject the different tissues to the same treatment and thus make the results more comparable. The anti-vimentin antibody was used to indicate the Sertoli cells, and anti SCP-3 antibody was used to indicate the spermatocytes. cPGES was weakly expressed in the Sertoli cells in mouse testes from infant to adult stages. During the juvenile to adult stages, cPGES was strongly expressed in the cytoplasm of spermatocytes and round spermatids. NC: negative control. Scale bars: 50μm.

coverslips, taking on a stretching polygonal shape. Sertoli cells were probed with anti-cPGES and anti-vimentin antibodies. FITP-labeled secondary antibody was used to monitor the Sertoli cell specific vimentin; meanwhile, Phalloidin-Texas Red-labelled secondary antibody was used to monitor cPGES. cPGES signal was mainly observed in the cytoplasm of the Sertoli cells and was concurrent with the presence of vimentin (Figure 3A).

The separated germ cells, including spermatocytes and round spermatids isolated from adult mouse testes, were examined by a similar method with anti-cPGES antibody and anti SCP-3 antibody. FITP-labeled secondary antibody was used to monitor the spermatocyte-specific SCP-3 protein. As shown in Figure 3B, the spermatocytes, which were larger in size, were stained by SCP-3 antibody, while round spermatids, which were relative small, were not stained. cPGES immuno-signal was seen in the cytoplasm of both spermatocytes and round spermatids. The results indicate that the Sertoli cells, spermatocytes, and round spermatids constitutively express cPGES and were in accordance with the immunohistochemistry results.

In the Western blotting analysis parallel to the immunofluorescence experiment, the Sertoli cells derived from infant or adult mice and spermatocytes and round spermatids isolated from adult mice were lysed respectively. Then the expression level of cPGES in these cells was analyzed. The results showed that cPGES was predominantly expressed in spermatocytes and round spermatids, with a similar level in the two types of cells. The level of cPGES in the Sertoli cells was about 15% compared with that in spermatocytes. No significant difference of cPGES level between infant and adult mouse Sertoli cells was found (Figure 4).

4.4. Expression of cPGES in mouse male genital organs

We also compared the expression level of cPGES in male mouse genital organs by Western blot analysis. The level of cPGES was quantified by relative band intensity which was normalized to β-actin levels. The data showed that cPGES was highly expressed in the testis, epididymis, and seminal vesicles and moderately expressed in vas deferens (Figure 5).

5. DISCUSSION

The amounts of prostaglandins in human semen are much higher than elsewhere in the body and PGE2 is one of the most common prostanoids in the seminal fluid of various animals (12-16). Previous works reported conflicting results about the source and the concentration of seminal prostaglandins. Some reports claimed that it is the seminal vesicle, not the testis, that is responsible for prostaglandin synthesis and secretion (12-14). However, other experiments revealed that the remaining parts of the male reproductive tract, including the testis, synthesize and secret prostaglandin (15, 25, 27).

Three PGE2 terminal synthetic enzymes were identified in the male reproductive tract. Among them, mPGES-1 was detected only in Leydig cells in mouse testes and in epithelial cells of the epididymis, vas deferens, and seminal vesicles (29), while mPGES-2 was restricted to the epithelial cells of the epididymis, vas deferens, and seminal vesicles. In the testis, mPGES-2 was abundant in the primary and secondary spermatocytes and spermatids and was very weak in spermatogonia, Sertoli cells, and Leydig cells (30).

In this study, we focused on the distribution and relative content of cPGES in the mouse testis during

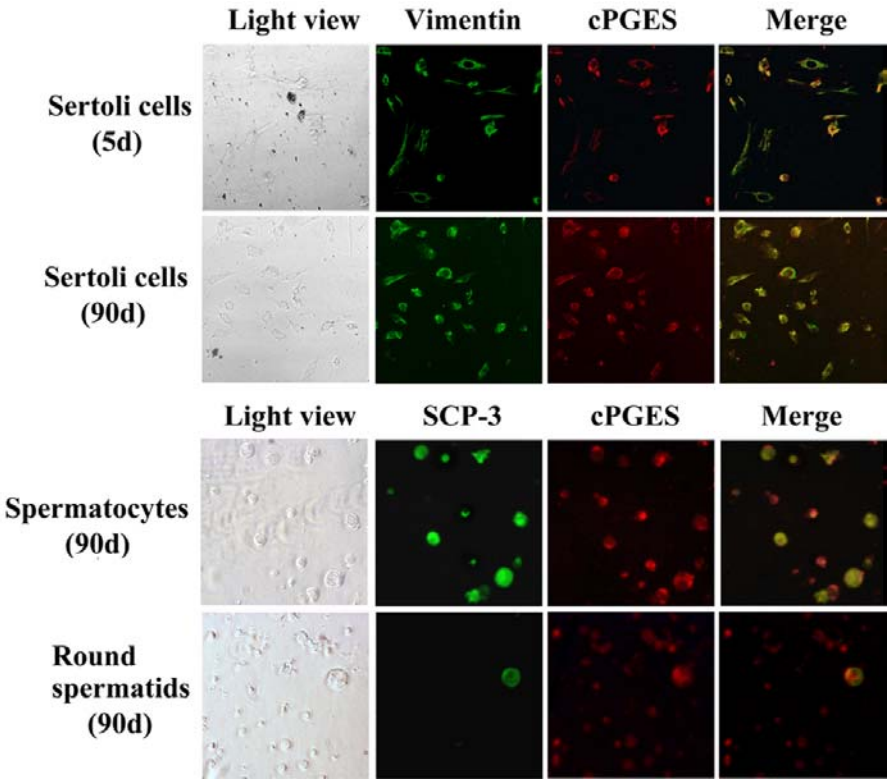

Figure 3. Immunofluorescent localization of cPGES in the isolated Sertoli cells and germ cells. (A) Sertoli cells isolated from 5- and 90-day mice were marked by anti-vimentin antibody, which was a specific marker of the Sertoli cells. These cells were also stained by anti-cPGES antibody. (B) Most cells in the spermatocyte fraction isolated from adult mice were marked by anti-SCP-3 antibody, which was a specific marker of the spermatocytes. These cells were also stained by anti-cPGES antibody. Most cells in the spermatid fraction isolated from adult mice were stained by anti-cPGES antibody, but not by anti-SCP-3 antibody.

postnatal development. In mice one day after birth, testicular seminiferous epithelium is composed of primordial germ cells and Sertoli cells. At the 7th and 14th days after birth, spermatogonia and prophase primary spermatocytes appear, respectively. Round spermatids form at the 21st day, and mature sperms form at the 35th day after birth, thus initiating the seminiferous epithelium cycle (36, 37). Our results showed that the expression of cPGES was weak in mouse testes 5 days after birth and increased through 10 days and 15 days. At the 20th and the 30th days, the cPGES expression in testes reached the level of adult mice (three months after birth). The results indicated that the expression of cPGES was obviously age-dependent (Figure 1). Furthermore, the localization and relative amount of cPGES in seminiferous tubules were determined by immunohistochemistry. In the 5- to 10-day-old mouse seminiferous tubules sections, cPGES immune signal was detectable in the Sertoli cells, which were vimentin positive and SCP-3 negative. In the 15- to 20-day-old mouse testis sections, meiotic germ cells, such as primary and secondary spermatocytes and the subsequent round spermatids, gradually appeared. In all of these germ cells cPGES was present, with the highest levels in spermatocytes and round spermatids. This may be responsible for the substantial cPGES increase directly in juvenile testes. At the 30th day, all types of germ cells except for sperm were present in the testis sections, and the cPGES expression pattern in testes seemed to be identical as compared with adult mice, in which cPGES was mainly localized to the spermatocytes and round spermatids, and Sertoli cells kept their expression of cPGES at a similar amount compared with that of 5- to10-day-old mice (Figure 2). The cPGES expression pattern in seminiferous tubules revealed by histochemistry in this study was in accord with the in situ hybridization results previously reported (31). In the Leydig cells of all stages examined, cPGES immune signal was comparable to, or relatively lower than, that in Sertoli cells (Figure 2), suggesting that testicular Leydig cells are not the main cells responsible for the synthesis of PGE2.

Immunofluorescent staining and Western blotting analysis on isolated Sertoli cells, spermatocytes, and round spermatids were employed to verify the histochemical cPGES expression patterns. The results showed that cPGES was constitutively expressed in Sertoli cells derived from infant or adult mice. No significant difference of cPGES level between infant and adult mouse Sertoli cells was found. cPGES was predominantly expressed in spermatocytes and round spermatids isolated from adult mice, with a relatively higher level in spermatocytes than in round spermatids. The level of cPGES in the Sertoli cells was only about 15% compared with that in spermatocytes (Figure 3, 4).

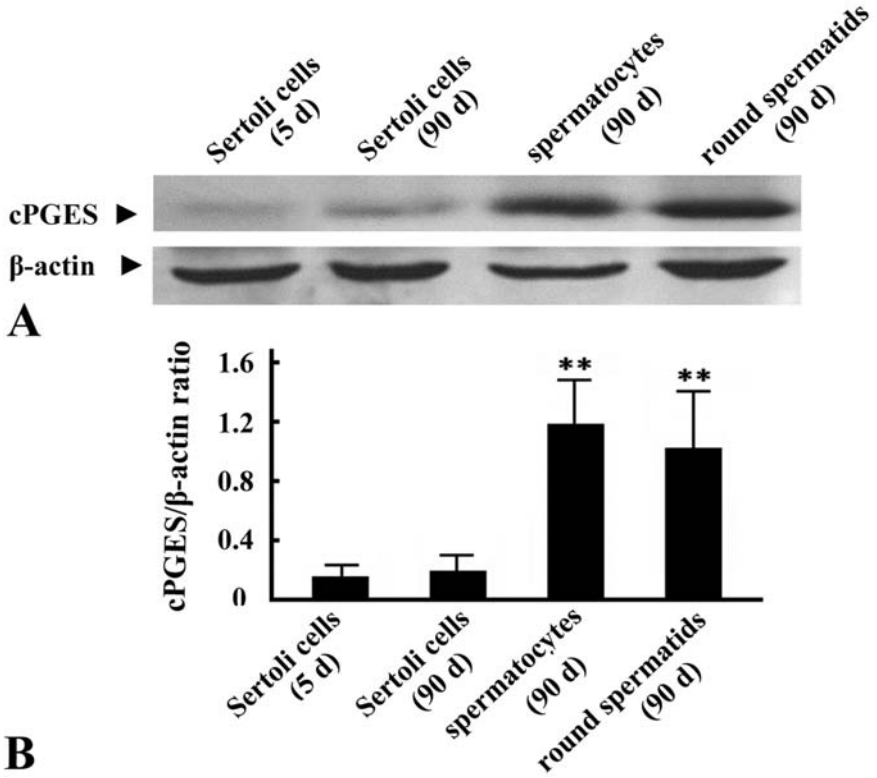

Figure 4. Comparison of cPGES expression in isolated Sertoli cells, spermatocytes, and round spermatids. (A) Sertoli cells isolated from 5- and 90-day mice and spermatocytes and round spermatids isolated from 90-day mice were examined by Western blot analysis. A representative immunoblot of cPGES and the protein loading control β-actin were shown. (B) The relative expression level of cPGES was measured by cPGES /β-actin ratio. Results are the mean±S.E. from four independent experiments. **$p < 0.01$, versus 5-day mouse Sertoli cells.

Previous studies revealed that there is an age-dependent alteration of PGE2 in the epididymis and vas deferens of the rat (38). The PGE2 peak in the epididymis, especially in the epididymis cauda, coincides with the peak of plasma FSH, the maturation of spermatozoa in the testis, and the arrival of testicular fluid and spermatozoa in the epididymis, suggesting that the PGE2 synthesis in the male reproductive tract is associated with the feedback regulation of FSH (38). Cooper et al reported that Sertoli cells synthesize prostaglandins and FSH enhances prostaglandin production. Furthermore, a single IV injection of PGE2 into male rats aged 30-35 days produces an increase of serum FSH (39). Hence, we hypothesize that the expression of a small amount of cPGES in Sertoli cells in the juvenile mouse testis provides a basic PGE2 level in seminiferous tubules, which is beneficial to the proliferation of spermatogonia during the early development of seminiferous epithelium. As spermatogenesis starts, germ cells, especially the spermatocytes and spermatids, express cPGES in a large amount. In adult mice, PGE2 synthesis in spermatogenic cells becomes the main synthetic pathway and source of PGE2 in seminiferous tubules, which may make the PGE2 level in seminiferous tubules easily couple with the spermatogenic cycle. In this way, the tubular fluid PGE2 content can match the number of sperms and meet the requirement of germ cell development, sperm activity, feedback regulation of FSH, and other physiological processes on the PGE2 concentration.

During sperm transport from seminiferous tubules to the epididymis, PGE2 generated from seminiferous epithelium cells directly stimulates the shrinking of the peritubular myoid cells to promote liquid delivery. Similarly, a high concentration of PGE2 is essential in the processes of sperm maturation and semen transport. By stimulating smooth muscle contraction, PGE2 facilitates the semen transport through the epididymal duct and vas deferens, as well as the seminal vesicle and other gland contents' secretion into the semen (29). However, given the short half-life of PGE2 (40) and the long time-span of sperm maturation in the epididymis, the maintenance of a certain concentration of PGE2 in semen requires constant synthesis and replenishment of PGE2. Several previous reports verified the expression of COX-1, COX-2, and mPGES in epididymis, vas deferens, and seminal vesicles (29, 30). Our results show, for the first time, that cPGES is also expressed in these tissues. Thus, both cPGES and mPGES may be involved in the synthesis of PGE2 at the rear end of male reproductive tract and contribute to the high concentration of PGE2 in semen.

It is generally considered that the COX-2 and mPGES-1-based PG synthesis system promotes the delayed

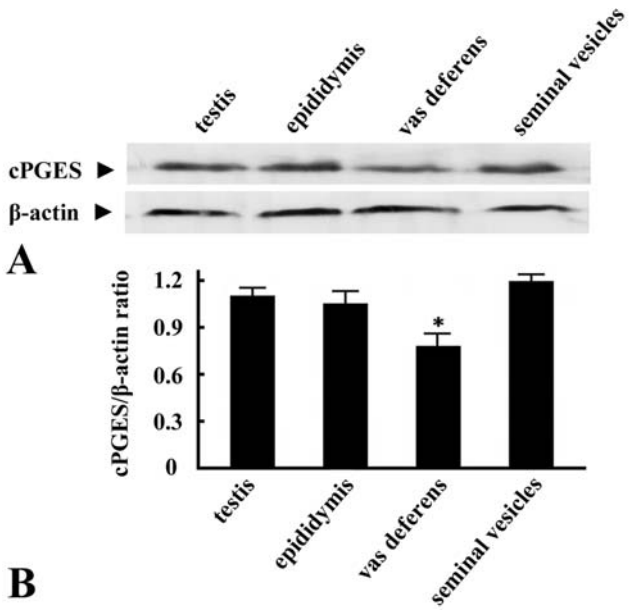

Figure 5. Expression of cPGES in mouse male genital organs. (A) The four regions of genital organs, including testis, epididymis, vas deferens, and seminal vesicles, were examined by Western blot analysis. (B) The relative expression level of cPGES was measured by cPGES /β-actin ratio. Results are the mean±S.E. from four independent experiments. *$p < 0.05$, versus testis.

PG synthesis response induced by proinflammatory stimuli (11). Obviously, the constant high concentration of PGE in semen should not, at least not primarily, be attributed to this synthesis system. However, the constitutively expressed cPGES and COX-1-based immediate PG synthesis system seems to be a more reasonable explanation.

It should be noticed that cPGES shares a very similar expression pattern in adult mouse testis with mPGES-2 (30). Previous reports demonstrated that mPGES-2 functionally couples to both COX-1 and COX-2 in the immediate and delayed response, with a modest COX-2 preference (30). The biological significance of the colocalization of these two terminal PGE2 synthases in the germ cells and Sertoli cells needs further investigation. It has been reported that PGE2 concentration decreased in cPGES knock-out mice (41), suggesting that the cPGES function can not be completely compensated by mPGES, although they are all associated with PGE2 biosynthesis. Therefore, the three PGESs may play distinct roles and participate in different physiological events (30, 42).

6. ACKNOWLEDGEMENTS

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