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Inhibin B Regulating Follicle-Stimulating Hormone Secretion During Testicular Recrudescence in the Male Golden Hamster

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ABSTRACT: In the present study, to clarify whether inhibin affects follicle-stimulating hormone (FSH) secretion in the recrudescence of the male golden hamster, we used a recently developed specific enzyme-linked immunosorbent assay (ELISA) in order to measure 2 forms of inhibin molecules: inhibin B and inhibin pro-αC. In addition, we used the radioimmunoassay (RIA) to measure immunoreactive (ir-)inhibin, FSH, luteinizing hormone (LH), and testosterone. And finally, we used the proliferating cell nuclear antigen (PCNA) and computer-assisted sperm motion analysis (CASA) methods to ascertain how well spermatogenesis and sperm motility recover from the photoinhibition caused by exposure to a short-day (SD; 10-hour light:14-hour dark) photoperiod. Animals were exposed to SD for 15 weeks, and then their testes were checked carefully and found to be completely regressed. Thereafter, those animals were transported to a long-day (LD; 14-hour light:10-hour dark) photoperiod. Sampling was carried out at weeks 0 (exposed SD 15 weeks), 1, 2, 4, 6, 8, and 10. Plasma FSH rapidly increased and reached peak levels 2 weeks after transferral to the LD photoperiod and then declined to normal LD levels at week 6. Circulating ir-inhibin, inhibin B, and inhibin pro-αC rose to normal LD levels by week 4. A highly significant inverse correlation was observed between plasma FSH and inhibin B but not between FSH and either ir-inhibin or inhibin pro-αC. Plasma testosterone recovered to normal LD levels within 1 week. Sperm motility parameters were low until week 2 and recovered to normal LD levels from weeks 4 to 10. PCNA-labeled cells were confined to the spermatogenic cells of the seminiferous tubules, though Leydig and Sertoli cell nuclei were never stained for PCNA during the period studied. The number of pachytene spermatocytes and the diameter of seminiferous tubules increased in a time-dependent manner after transferral from SD to LD. In conclusion, these results suggest that 1) secretion of inhibin B may be stimulated by an early rise in FSH; 2) inhibin B suppresses FSH secretion from weeks 2 to 10, after transferral to the LD photoperiod; and 3) testes recrudescence is based on the increase in the number of sperm cells instead of the increase in the number of Sertoli and Leydig cells of the male golden hamster.

Key words: Photoperiod, proliferating cell nuclear antigen, sperm motion.

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The golden hamster, unlike most commonly used laboratory rodents, has been an invaluable model for studies of the seasonal pattern of reproduction. In male hamsters, exposure to a short photoperiod has been found to induce morphological and functional regression of the reproductive system (Berndtson and Desjardins, 1974; Turek et al, 1975; Goldman et al, 1981). Continuous exposure to inhibitory photoperiods causes hamsters to become insensitive to the light regimen, probably because of the interruption of nocturnal pineal melatonin synthesis (Lerchl and Nieschlag, 1992). Conversely, it has been demonstrated that when hamsters are transferred from a short-day (SD) photoperiod to a long-day (LD) photoperiod, plasma follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, and immunoreactive (ir)-inhibin return to normal levels, inducing the recovery and reestablishment of testicular functions (Berndtson and Desjardins, 1974; Turek et al, 1975; Matt and Stetson, 1980; Goldman et al, 1981; Simpson et al, 1982; Milette et al, 1988; Kirby et al, 1993). However, it is evident that the rise in plasma FSH precedes any change in plasma levels of LH and testosterone during photoperiod-induced spontaneous testicular recrudescence in male golden hamsters (Turek et al, 1975; Milette et al, 1988; Kirby et al, 1993; Donham et al, 1994). It has been suggested that an early rise in FSH secretion is primarily dependent upon endogenous gonadotropin-releasing hormone (GnRH) release (Meredith et al, 1998).

It is well known that inhibin is a heterodimeric protein

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consisting of an α subunit and one of 2 β subunits. Two related forms of inhibin, inhibin A (α/βA) and inhibin B (α/βB), are secreted into the circulation from the gonads and inhibit pituitary FSH secretion (Ling et al, 1985; Miyamoto et al, 1985; Rivier et al, 1985; Robertson et al, 1985; Vale et al, 1986). A recently developed specific immunoassay has enabled the measurement of plasma levels of dimeric inhibins and provided evidence that inhibin B is an important physiological form of inhibins in male golden hamsters (Jin et al, 2001b), as well as in other males: male Gottingen miniature pigs (Jin et al, 2001a), men (Illingworth et al, 1996), male monkeys (Foppiani et al, 1999; Ramaswamy et al, 2000), male chimpanzees (Kondo et al, 2000), and male rats (Sharpe et al, 1999). It has also been suggested that in men, inhibin B regulates FSH secretion (Anawalt et al, 1996; Illingworth et al, 1996; Nachtigall et al, 1996; Seminara et al, 1996). However, the existence of negative feedback of inhibin B on FSH secretion during photoperiod-induced testicular recrudescence in golden hamsters has been disputed and is yet to be elucidated. Kirby et al (1993) suggested that testicular ir-inhibin secretion may not be directly related to circulating FSH levels during recrudescence in the golden hamster.

The proliferating cell nuclear antigen (PCNA), a 36-kd acidic nuclear protein that has been very highly conserved in the process of evolution, is a cell proliferation marker currently drawing attention. PCNA is known to function as a cofactor for DNA polymerase δ, with a biological half-life of longer than 20 hours (Bravo and Macdonald-Bravo, 1987). PCNA is required for both DNA replication and DNA repair (Shivji et al, 1992; Xiong et al, 1992). It is synthesized primarily during the G1 phase of the cell cycle and reaches its maximum levels during the S phase (Hofstadter et al, 1995). Given adequate fixation and tissue processing, the results of PCNA immunohistochemistry directly reflect the proliferative status of the cells and the fact that PCNA-labeled nuclei are observed during the G1 to the S phase of the cell cycle (Morita et al, 1994). The localization of PCNA can be used to assess the proliferative status of renewing spermatogonia and to analyze the proliferative activity of the seminiferous epithelium of rodents and non-human primates (Schlatt and Weinbauer, 1994) and men (Garrido et al, 1992). Thus, PCNA expression levels in the cell nuclei are indicative of the proliferating activity of the renewing spermatogenic cells.

Therefore, in the present study, we tested the hypothesis that triggering the secretion of inhibins and the resulting increased levels of inhibins subsequently affect the FSH during the photoperiod-induced testicular recrudescence in the male golden hamster. Furthermore, renewing spermatogenic cell proliferating activity was evaluated by PCNA. and sperm motility characteristics were also monitored by a computer-assisted sperm analysis system (CASA) to ascertain how well the spermatogenesis recovers from the photoinhibition caused by exposure to an SD photoperiod.

Materials and Methods

Animals and Blood Samples

Adult male golden hamsters (*Mesocricetus auratus*) (3 months old) were used in the present study. The experimental design was based on a previous study by Donham et al (1994), who reported that the SD photoperiod-induced testicular regression was completed by 15 weeks. Thus, in the present study, experimental animals were kept under an SD photoperiod (10-hour light:14-hour dark cycles; lights on at 0900 hours) for 15 weeks. Upon complete regression of the testes at 15 weeks, the animals were transferred to an LD photoperiod (14-hour light:10-hour dark cycles; lights on at 0500 hours). The sampling was carried out at weeks 0, 1, 2, 4, 6, 8, and 10 (week 0 is the 15th week of the SD photoperiod). At each time point, 5 animals were killed by decapitation between 0900 and 1100 hours. Blood was collected, stored in ice, and centrifuged at 1700 × *g* for 15 minutes at 4°C. Plasma was separated and stored at −20°C until assayed for inhibins, LH, FSH, and testosterone. Control animals of the same age were housed in groups of 6 to 8 animals per cage in a room with controlled conditions of temperature, humidity, and lighting (14-hour light:10-hour dark cycles; lights on at 0500 hours). Food and water were available ad libitum. All experimental procedures involving animals were carried out in accordance with requirements established under the Guide for the Care and Use of Laboratory Animals by the Tokyo University of Agriculture and Technology. The experiment was repeated, and the data were found to be reproducible.

Radioimmunoassays for FSH, LH, Testosterone, and ir-Inhibin

Plasma concentrations of FSH and LH were measured using National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) radioimmunoassay (RIA) kits for rat FSH and LH, as described previously (Bast and Greenwald, 1974). The antisera used were anti-rat FSH (S-11) and LH (S-10). Results were expressed in terms of NIDDK rat FSH (RP-2) and LH (RP-2). The intra- and interassay coefficients of variation were 4.4% and 14.6% for FSH and 6.7% and 8.9% for LH, respectively.

Plasma concentrations of ir-inhibin were measured by a double-antibody RIA, as described previously (Hamada et al, 1989). The antiserum used was raised in rabbits against bovine inhibin (TNDH-1). Purified bovine 32-kd inhibin was used as the standard. The assay system does not distinguish dimeric inhibin from α subunit monomer. The intra- and interassay coefficients of variation were 8.8% and 14.4%, respectively.

Plasma concentrations of testosterone were determined by a double-antibody RIA system using ^{125}I-labeled radioligand, as described previously (Taya et al, 1985). Antiserum against testosterone was kindly supplied by Dr G. D. Niswender (Animal Reproduction and Biotechnology Laboratory, Colorado State

University, Fort Collins, Colo) (Gay and Kerlan, 1978). The intra- and interassay coefficients of variation were 6.3% and 7.2%, respectively.

Enzyme-Linked Immunosorbent Assay

Plasma concentrations of inhibin B and inhibin pro-αC were determined using enzyme-linked immunosorbent assay (ELISA) kits (Serotec Ltd, Oxford, United Kingdom). Inhibin A was not measured because we have previously reported that plasma inhibin A is not detectable in male golden hamsters (Jin et al, 2001b). We have validated and reported these inhibin dimer-specific assays for male golden hamsters (Jin et al, 2001b).

Tissue Processing

In each case, the right testis, including the epididymis, the seminal vesicle, and the coagulating gland complex, was weighed, and sperm from the right epididymis were used for sperm motion analyses. Testicular tissue samples were immediately fixed in freshly prepared 4% paraformaldehyde (Sigma Chemical Co, St Louis, Mo) in 0.05 M phosphate-buffered saline, pH 7.4, and embedded in paraffin. The paraffin-embedded testicular tissues were serially sectioned at 6-μm thickness and mounted onto poly-L-lysine (0.01% [wt/vol]) (Sigma) coated slide glasses (Dako Japan Co, Kyoto, Japan) for use in immunohistochemistry.

Immunohistochemistry for PCNA

After being deparaffinized with xylene, the tissue sections were subjected to antigen retrieval by autoclaving in 0.01 M sodium citrate buffer, pH 6.0, at 121°C for 15 minutes. Sections were then incubated in 6% H_2O_2 in methanol at room temperature for 1 hour, followed by 0.5% casein-Tris saline (0.05 M Tris-HCl with 0.15 M NaCl, pH 7.6; CTS) at 37°C for 1 hour, to quench nonspecific staining. Then, the tissue sections were incubated at 37°C for 16 to 18 hours with a monoclonal antibody raised against PCNA (Biomeda, Forster City, Calif) at a dilution of 1:200 in CTS. After incubation with the antibody, sections were treated with 0.25% (vol/vol) biotinylated goat anti-mouse secondary antibody (Elite ABC kit, Vector Laboratories, Burlingame, Calif) in CTS at 37°C for 1 hour. These sections were subsequently incubated with 2% (vol/vol) avidin-biotin complex (Elite ABC kit) in CTS at 37°C for 30 minutes. The reaction products were visualized by treatment with 0.025% (wt/vol) 3.3′-diaminobenzidine tetrachloride (DAB; Sigma) in 100 mM Tris-buffered saline containing 0.01% H_2O_2 for 1 to 30 minutes.

Computer-Assisted Sperm Mobility Analyses

The sperm motility parameters were obtained using the C. IMAGING CASA system. Sperm from the cauda epididymis were incubated at 37°C for 3 minutes in 0.01 M medium buffer, pH 7.2. The medium buffer was made up of 59.8 mg of HEPES (Dojindo, Kumamoto, Japan), 982 mg of medium 199 (Biocell, Carson, Calif), 500 mg of bovine serum albumin (Sigma), and 220 mg of $NaHCO_3$ (Wako, Osaka, Japan) dissolved in 100 mL deionized water. After the sperm were incubated in medium buffer at 37°C, an aliquot of this solution was diluted 10- to 20-fold, and 10 μL was placed into the microcell-HAC chamber, which has a depth of 50 μm (Conception Technologies, San Diego, Calif). Analyses of motility characteristics were performed on at least 200 cells for each sample. Sperm motion, as viewed on an Olympus microscope (4×, pseudodark-field optics) with a stage warmer (37°C) (MP-10DM; Kitazato Supply Co, Kitazato, Japan), was analyzed using the C. IMAGING system. The C. IMAGING system settings were as follows: frames analyzed, 15; framing rate, 30; maximum velocity, 1200 μm/s; threshold velocity, 45 μm/s; minimum linearity for ALH (amplitude of lateral head displacement), 3.5; pixel scale, 3.26 mm/pixel; maximum average number of cells/field, 30; and cell size range, 350 to 1600 pixels. The following characteristics were analyzed: percentage of motile spermatozoa, curvilinear velocity (total distance traveled divided by total time the cell was tracked), straight velocity (straight-line distance), mean ALH (deviation of the sperm head from the mean trajectory), max ALH (the maximum amplitude of lateral head displacement), linearity (ratio of the straight-line distance to the actual tracked distance), and percentage of circular cells.

Histological Analysis

The PCNA-labeled germ cells were counted under the microscope (Nikon, Tokyo, Japan). The diameters of 20 round tubules per animal were measured.

Statistics

A one-way analysis of variance was performed. Significance was determined by the Duncan multiple range test (Steel and Torrie, 1960). Correlation analysis between inhibins and FSH was performed using the Pearson method. All data are presented as the mean plus or minus the standard error of the mean. Differences were considered significant when P was less than .05.

Results

Changes in Weight of Reproductive Organs

Body weights (BWs) were not different between the treatment and control groups during the experimental period (Figure 1A). However, testes in the hamsters exposed to an SD photoperiod for 15 weeks displayed more than a 10-fold reduction ($P < .0001$) in weight. Relative testes (testes/BW) and epididymal (epididymal/BW) weights were significantly ($P < .001$) low in the treatment group compared with those in the control group until the fourth (testes) and sixth (epididymal) weeks. The weight of the testes and epididymis then began to recover and reached normal LD levels by 6 and 8 weeks, respectively (Figure 1B and D). However, the relative weight of the seminal vesicle (seminal vesicle plus coagulating gland complex) had not recovered to normal LD levels by 10 weeks (Figure 1C).

Changes in Plasma Concentrations of FSH, LH, ir-Inhibin, Inhibin B, Inhibin pro-αC, and Testosterone

The changes in plasma concentrations of reproductive hormones are shown in Figure 2. Rapid increases in con-

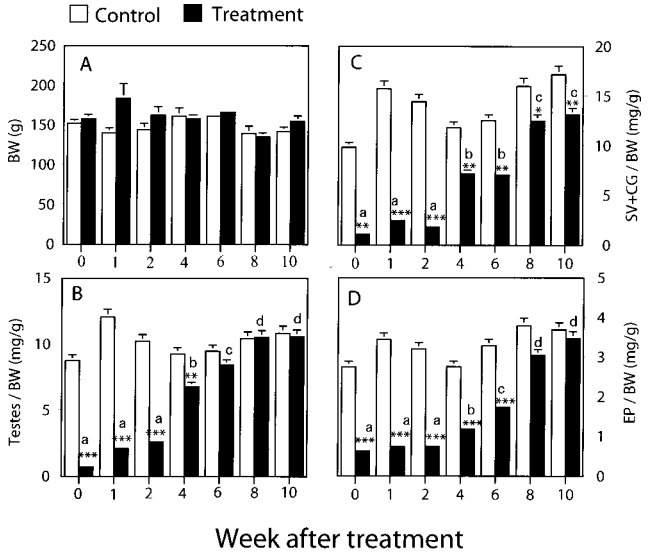

Figure 1. Changes in **(A)** body weights (BW), relative weights of **(B)** testes, **(C)** the seminal vesicle and coagulating gland complex (SV + CG), and **(D)** epididymis (EP). Each point represents the mean plus or minus the standard error of the mean from 5 animals. $*P < .05$, $**P < .01$, $***P < .0001$, compared with the control group; different characters indicate a significant difference ($P < .05$) in each graph among the treatment group.

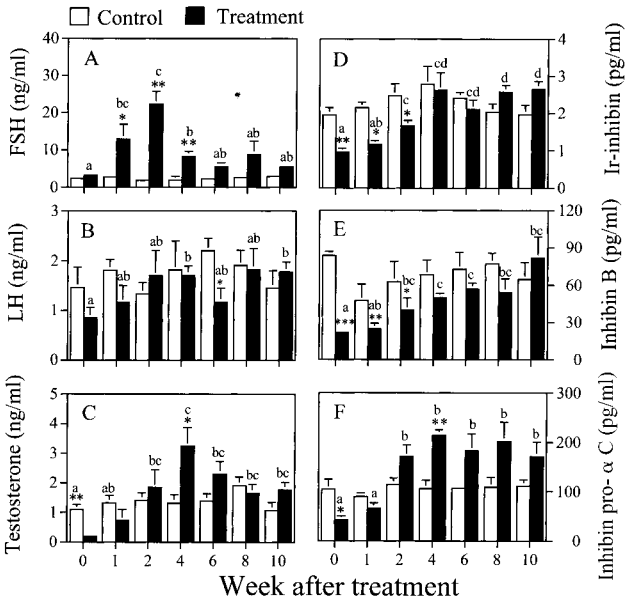

Figure 2. Changes in plasma concentrations of **(A)** follicle-stimulating hormone (FSH), **(B)** luteinizing hormone (LH), **(C)** testosterone, **(D)** immunoreactive (ir)-inhibin, **(E)** inhibin B, and **(F)** inhibin pro-αC during a 10-week period after the transfer from short-day (SD) to long-day (LD) photoperiods in male golden hamsters. Each point represents the mean plus or minus the standard error of the mean from 5 animals. $*P < .05$, $**P < .01$, $***P < .0001$, compared with the control group; different characters indicate a significant difference ($P < .05$) in each graph among the treatment group.

centrations of plasma FSH occurred at 1 week after transferral from an SD to an LD photoperiod and reached peak levels (22 ng/mL) ($P < .01$) at week 2, followed by a rapid decline to normal LD levels (1.5–3 ng/mL) at week 6 (Figure 2A). In contrast, basal concentrations of plasma LH steadily increased after transferral from the SD to the LD photoperiod and were significantly high at weeks 4 and 10 compared with week 0, although these levels were not significantly different from those in the control group (Figure 2B). Plasma concentrations of testosterone were significantly ($P < .05$) low at week 0 in the treatment group compared with those in the control group. These concentrations then gradually increased and reached peak levels at week 4 before declining to normal LD levels (Figure 2C). Plasma concentrations of ir-inhibin (Figure 2D) and inhibin B (Figure 2E) began to increase at week 2 and reached normal LD levels at week 4. Plasma concentrations of inhibin pro-αC were significantly ($P < .05$) low at week 0 in the treatment group compared with those in the control group, but they then began to increase at week 1 and remained at higher levels than those in the control animals from weeks 2 to 10 (Figure 2F). Plasma FSH and inhibin B concentrations were inversely correlated ($r = -0.56$, $P < .01$) during the period from weeks 2 to 10 (Figure 3). However, neither ir-inhibin nor inhibin pro-αC was correlated with plasma FSH (data not shown).

Changes in Sperm Motility Parameters

No sperm were available in the cauda epididymis for motile parameter analysis until week 2, but 1 of the 5 animals had some sperm at week 2 (data not shown). The percentage of motile spermatozoa was abruptly increased at week 4 and reached normal LD levels at week 6 (Figure 4A). At week 10, the percentage of motile sperm was reduced slightly but significantly ($P < .05$) in the treatment group compared with that in the control group. The ALH mean (Figure 4B) and ALH max (Figure 4C) in the treatment group increased abruptly at week 4, although

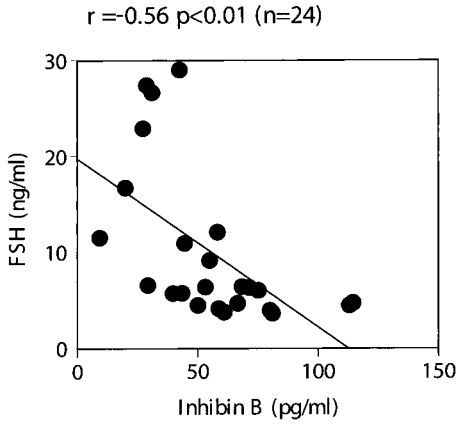

Figure 3. Relationship between plasma follicle-stimulating hormone (FSH) and inhibin B concentrations during the period from weeks 2 to 10 after the transfer from short-day (SD) to long-day (LD) photoperiods in male golden hamsters. There was an inverse correlation ($r = -0.56$, $n = 24$, $P < .01$) between these 2 hormones.

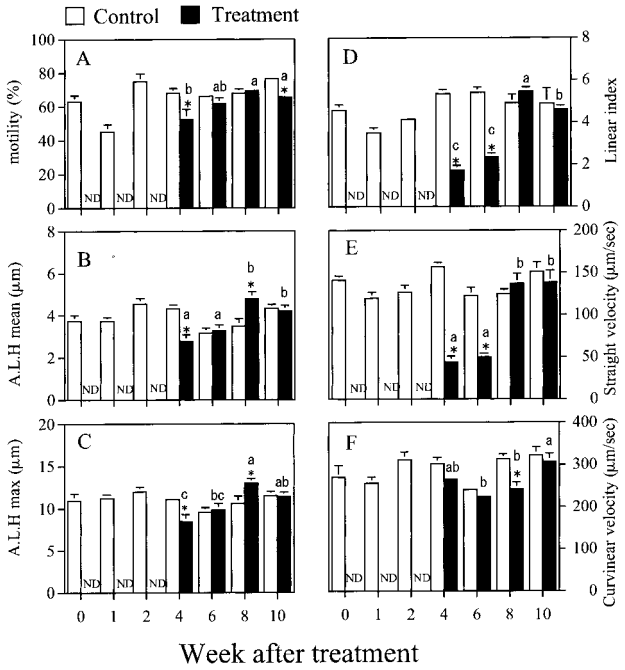

Figure 4. Epididymal sperm motility parameters: percentages of motile spermatozoa **(A)**, amplitude of lateral displacement (ALH) mean **(B)**, ALH max **(C)**, linear index **(D)**, straight velocity **(E)**, and curvilinear velocity (F) during a 10-week period after the transfer from short-day (SD) to long-day (LD) photoperiods in male golden hamsters. No sperm were detected in the cauda epididymis until 4 week of the LD photoperiod. Each point represents the mean plus or minus the standard error of the mean from 5 animals. $*P < .05$, $**P < .01$, $***P < .0001$ compared with the control group; different characters indicate a significant difference ($P < .05$) in each graph among the treatment group.

these levels were still significantly low compared with those in the control groups; however, this was followed by further increases to the control levels by week 6. Linear index (Figure 4D) and straight velocity (Figure 4E) were still significantly ($P < .05$) low in the treatment group at weeks 4 and 6 compared with those in the control animals. These parameters recovered to those of the control levels at week 8. Curvilinear velocity (Figure 4F) abruptly recovered to normal LD levels at week 4.

Immunohistochemistry for PCNA

In order to better visualize cell types, the sections were stained with hematoxylin and eosin at weeks 0, 2, 4, 6, and 8 in the treatment group (Figure 5A, B, C, D, and E, respectively) as well as in the control group (Figure 5F). At weeks 0 and 2, the seminiferous tubules contained primarily Sertoli cells and spermatogonia, but spermatocytes and round spermatids were also occasionally seen (Figure 5A and B). The testes of animals in the treatment group at weeks 0 and 2 showed dilated seminiferous tubules with small-sized spermatogenic cells, Sertoli cells, and Leydig cells. However, there were no mature spermatids contained within the seminiferous tubules. In early recrudescence, there was an increase in the number of germ cells in the basal compartment. The increase in the cell volume of Sertoli and Leydig cells, as well as the number of germ cells in the seminiferous tubules, appeared to be time-dependent during weeks 4, 6, and 8 (Figure 5C, D, and E, respectively). The testes in animals exposed to the SD photoperiod recovered to normal LD appearance at 10 weeks.

Germ cells from the testes were positively stained for PCNA at weeks 0, 2, 4, 6, and 8 in the treatment group (Figure 5M, N, O, P, and Q, respectively) and in the control group (Figure 5R) in the male golden hamsters. PCNA was positively stained in early spermatogenic cells but not in the nuclei of Sertoli and Leydig cells at weeks 0 and 2 (Figure 5M through R). The increase in the size of immunopositive cells and in the number of spermatogenic cells was time-dependent. Relatively large nuclei of spermatogenic cells were positively stained with anti-PCNA antibody in the testes of the LD controls (Figure 5R). Sections incubated with normal mouse plasma instead of primary antibody did not show any immunopositive staining at weeks 0, 2, 4, 6, and 8 in either the treatment group (Figure 5G, H, I, J, and K, respectively) or the control group (Figure 5L).

Testes Histological Analysis

The numbers of pachytene spermatocytes and the diameter measurements of seminiferous tubules are shown in the Table. Both sets of numbers increased in a time-dependent manner during testicular recrudescence. The number of pachytene spermatocytes increased to normal LD levels at week 4 and reached peak levels at week 8. The diameter of seminiferous tubules also increased but still did not recover to normal LD levels at week 4; however, this was followed by a further increase to supracontrol levels at weeks 6 and 8.

Discussion

This is the first study that describes in detail the changes in 3 forms of inhibins—ir-inhibin, inhibin B, and inhibin pro-αC—and indirectly indicates that inhibin B affects FSH secretion during testicular recrudescence in the golden hamster. Furthermore, the results of PCNA together with the histological analysis suggest that testicular recrudescence is based on the increase in the number of spermatogenic cells rather than the increase in the number of Sertoli and Leydig cells in the testes of the male golden hamster. The present study shows that plasma FSH is rapidly elevated after the transfer of animals from an SD to an LD photoperiod and that it then begins to decline at a time when plasma inhibin B is elevated, suggesting that inhibin B is the major regulator of plasma FSH concentrations during this time. This finding is also reflected in

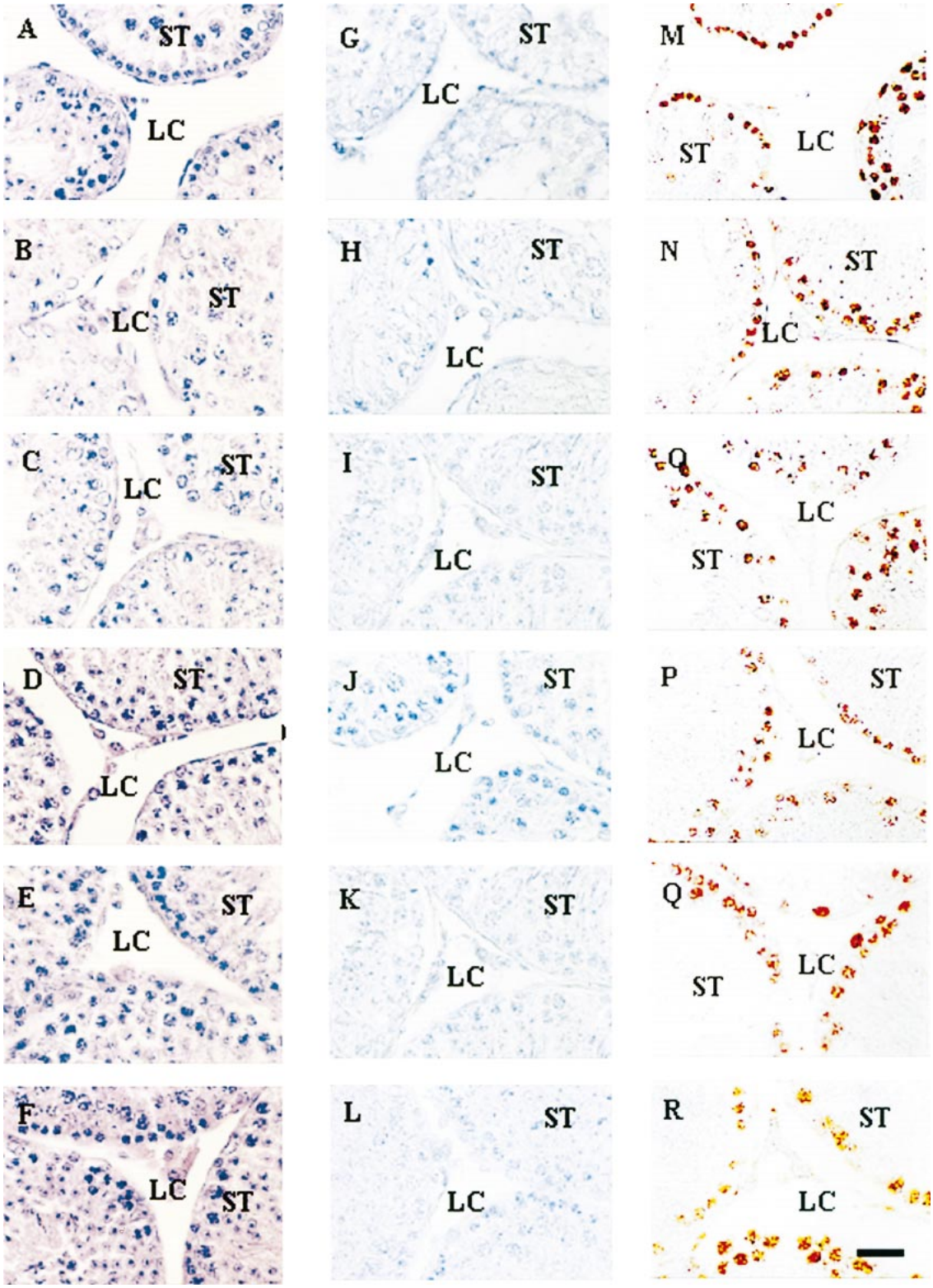

Figure 5. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) during a 10-week period after the transfer from short-day (SD) to long-day (LD) photoperiods in the testes of male golden hamsters. Germ cells at multiple stages of development were positively stained for PCNA at weeks 0 **(M)**, 2 **(N)**, 4 **(O)**, 6 **(P)**, and 8 **(Q)**. A positive staining of PCNA in the germ cells of the control animals, which were exposed to the LD photoperiod, is also given **(R)**. To better visualize cell types, the sections were stained with hematoxylin and eosin and are shown for weeks 0 **(A)**, 2 **(B)**, 4 **(C)**, 6 **(D)**, and 8 **(E)**, as well as for control animals **(F)**. Sections incubated with normal mouse plasma instead of primary antibody did not show any immunopositive staining at weeks 0 **(G)**, 2 **(H)**, 4 **(I)**, 6 **(J)**, or 8 **(K)**; the same was true for the control animals **(L)**. Bar = 20 μm.

*Histological analysis of spermatogenesis**

Group	Pachytene Spermatocytes	Tubular Diameter (μm)
0	13.33 ± 2.81A†	98.5 ± 3.19A
2	33.0 ± 3.15AB	166.0 ± 4.44B
4	43.4 ± 7.28BC	172.5 ± 5.93B
6	60.0 ± 6.63C	258.0 ± 5.88D
8	72.4 ± 3.47D	268.0 ± 5.97D
Control	60.6 ± 7.31C	228.25 ± 5.70C

* Shown are the means plus or minus the standard error (n = 5).
† Means with different letters in the same column are significantly different ($P < .05$; Duncan multiple range test).

a strong inverse correlation between these 2 hormones. The present study also demonstrates that gradual increases in plasma LH and testosterone occurring at the later stage of the LD photoperiod restore spermatogenesis, which has been adversely affected during exposure to the SD photoperiod.

Previous studies have suggested that in men, inhibin B regulates FSH secretion (Anawalt et al, 1996; Illingworth et al, 1996; Nachtigall et al, 1996; Seminara et al, 1996). We have also shown in our previous studies that inhibin B is the major circulating form of dimeric inhibins in the male golden hamster (Jin et al, 2001b) and in the Gottingen miniature pig (Jin et al, 2001a). Others have shown inhibin B as the major form of inhibins in other species as well, including male monkeys (Plant et al, 1997; Foppiani et al, 1999; Ramaswamy et al, 2000), male rats (Woodruff et al, 1996), and male chimpanzees (Kondo et al, 2000). It has been reported that inhibin B may have a physiological role in the development of spermatogenesis in the testes of adult male animals (Foppiani et al, 1999; Ramaswamy et al, 2000), though equine fetal testes (Tanaka et al, 2002) and ovine adult testes (McNeilly et al, 2002) secrete a large amount of inhibin A. In line with these findings, it is quite possible that there is inhibin B-FSH negative feedback during testicular recrudescence in the male golden hamster. Kirby et al (1993) did not observe any correlation between inhibin α and FSH levels during testicular recrudescence. We did not measure plasma inhibin A levels in the present study because we have shown in a previous study that plasma inhibin A is undetectable in the male golden hamster (Jin et al, 2001b).

In our present study, abrupt increases in plasma FSH levels preceded changes in plasma LH and testosterone after the animals were transferred from the SD to the LD photoperiod. Plasma FSH levels continued to rise through week 2 after transferral from the SD to the LD photoperiod, while there were gradual increases in basal plasma LH and testosterone. This result corroborates previous studies (Milette et al, 1988; Donham et al, 1994), which have reported that elevated FSH levels preceded spontaneous recrudescence of the testes in male golden hamsters. It also has been suggested that a rapid increase in plasma FSH after photostimulation is the primary signal for initiating testicular development in Djungarian hamsters (Milette et al, 1988). It was reported that the administration of exogenous FSH causes the regrowth of testes and complete spermatogenesis in photoinhibited Djungarian hamsters (Niklowitz et al, 1989). It is reasonable to suggest that FSH is important for seminiferous tubular development as well as for inhibin production by testes. On the other hand, gradual increases in basal plasma LH and testosterone levels were also observed in the present study. It is possible that the early elevation of FSH levels stimulates the induction of LH receptors in Leydig cells, thereby increasing the responsiveness of Leydig cells to endogenous and exogenous LH and thus stimulating testosterone production (Parvinen et al, 1984; Verhoeven and Cailleau, 1985). Niklowitz et al (1989) also reported that FSH alone initiated complete spermatogenesis, whereas LH alone induced full redifferentiation of Leydig cell function, resulting in increased testosterone production in the photoinhibited and hypophysectomized Djungarian hamsters. However, plasma inhibin concentrations continued to rise until week 4, during which time plasma FSH had fallen to low levels. Inhibin B production was apparently stimulated by an early rise in FSH, thus resulting in circulating levels sufficient to suppress FSH release. This finding shows further that there is a dynamic change in hormonal interrelationships.

The present study evaluated the localization of PCNA in the cell nuclei in order to identify cellular proliferating activity occurring during testicular recrudescence. Immunolocalization of PCNA has been widely used as a method for the detection of proliferating cells in tumors (Wada et al, 1993), developing tissues (Casasco et al, 1993), and testes (Schlatt and Weinbauer, 1994). In the present study, we have identified PCNA as being localized to the nuclei of early spermatogenic cells; in addition, we have found that the number and size of these immunopositive spermatogenic cells appear to increase in a time-dependent manner. In contrast, nuclei of both Leydig and Sertoli cells were not stained for PCNA. This result agrees with previous findings that both Leydig cells (Hikim et al, 1988; Sinha Hikim et al, 1993) and Sertoli cells (Hikim et al, 1988) increased in volume but not in number during testicular recrudescence in the male golden hamster. In line with these results, Liang et al (2001) reported that, in male monkeys, PCNA-immunopositive spermatogenic cells were increased in an age-dependent manner and that the positive staining for PCNA in Sertoli cell nuclei was observed in the testes of animals from the neonatal to the pubertal stage but not in adult animals. Together with the present histological analysis (shown in the Table), it is likely that the abrupt increase in plasma FSH and the steady increase in plasma LH, which occurred at an early stage of testicular recrudescence in the

golden hamster, were responsible for the increased volume of the Sertoli and Leydig cells. This rise in plasma FSH and LH levels possibly increases Leydig cell capacity to produce an increased amount of inhibins, because Leydig cells are the major source of inhibin B in the testes of the golden hamster (Jin et al, 2001b).

Although photoperiod-induced testicular recrudescence appears to begin at an early stage in the golden hamster, we could not detect any sperm until 2 weeks after transferral to the LD photoperiod. This suggests that the first wave of spermatogenesis apparently requires approximately 1 to 2 weeks after the transfer of animals from the SD to the LD photoperiod. It should be noted, however, that although sperm were detected in an animal in the treatment group at week 2, the profiles of sperm motility parameters were very low (data not shown). It should also be noted that in other animals, although sperm were present in the cauda epididymis after 4 weeks of normal LD exposure, in general, none of the parameters returned to normal values until about weeks 6 to 8.

In conclusion, the present results demonstrate the existence of a negative feedback of inhibin B on FSH secretion in male golden hamsters after transferral from an SD to an LD photoperiod. This feedback appears to begin functioning at an early stage of photoperiod-induced spontaneous testicular recrudescence. Plasma FSH as a primary signal, in combination with other reproductive hormones such as inhibin B, is likely to be the stimulus for the proliferation of various cells, leading to the reestablishment of the spermatogenic process.

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