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GnRH-A Induced Arrest of Spermiogenesis in Rats is Associated with Altered Androgen Binding Protein Distribution in the Testis and Epididymis

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ABSTRACT: This study examines the effects of a potent gonadotropin releasing hormone (GnRH)-antagonist (GnRH-A, Ac-D[2] Nal[1], 4-CL-D Phe[2], D-Trp[3], D-Arg[6], D-Ala[10]) upon the distribution of androgen binding protein (ABP) in serum, testis, and epididymis, and its relationship with the completion of spermatogenesis in Sprague-Dawley rats. After 2 weeks of daily injections of 10 μg/kg, 50 μg/kg, 100 μg/kg, or 500 μg/kg of GnRH-A, testicular ABP content was either unchanged or elevated ($P < 0.05$), and serum ABP levels were elevated ($P < 0.01$). Spermatogenesis was maintained in animals administered 10 μg/kg or 50 μg/kg GnRH-A, and epididymal ABP content remained unchanged. On the other hand, daily injections of 100 μg/kg or 500 μg/kg GnRH-A resulted in a significant decrease in epididymal ABP content ($P < 0.05$), and spermatogenesis was arrested at early spermiogenesis. After 4 weeks of GnRH-A administration, both testicular and epididymal ABP were decreased in a dose-dependent manner in animals receiving doses of 50 μg/kg or higher of GnRH-A. In order to evaluate the normalcy of the bidirectional release of ABP in GnRH-A treated rats, additional rats were given daily injections of 25 μg/kg or 250 μg/kg of GnRH-A for 2 weeks. Concentrations of ABP in interstitial fluid (ITF) and seminiferous tubular fluid (STF) remained unchanged, but serum ABP levels were significantly increased ($P < 0.05$) in rats administered 25 μg/kg GnRH-A. Qualitatively normal spermatogenesis was maintained and epididymal ABP content did not differ from that of control animals. In contrast, administration of 250 μg/kg GnRH-A resulted in a significant elevation of ABP concentration in both serum ($P < 0.01$) and ITF ($P < 0.05$), while ABP in STF remained unchanged. Spermatogenesis was arrested at early spermiogenesis and was associated with a marked decrease of epididymal ABP content ($P < 0.01$). These results demonstrate that disruption of spermatogenesis following high doses of GnRH-A was associated with abnormal distribution of ABP between the testis and epididymis, as well as elevated serum ABP. Despite the maintenance of qualitatively normal spermatogenesis and normal epididymal ABP content in rats administered low doses of GnRH-A, serum ABP was also elevated. The mechanisms responsible for these changes remain unknown, as does their possible involvement in the regulation of spermiogenesis.

Key words: GnRH-antagonist, Sertoli cell, spermatogenesis, androgen binding protein.

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Administration of gonadotropin-releasing hormone (GnRH) antagonists prevents normal interaction between endogenous GnRH and the pituitary (Rea et al, 1986), resulting in the suppression of gonadotropins and testosterone secretion. The subsequent decrease in intratesticular testosterone concentration is considered to be the cause for the subsequent regression of the seminiferous epithelium (Rivier et al, 1979; Debuljuk et al, 1983; Sundaram et al, 1984). Recent findings, however, that complete spermatogenesis can be maintained or restored in the presence of a testosterone concentration that is less than 25% of the normal concentration in testicular tissue or fluids in both intact rats (Cunningham and Huckins, 1979; Zirkin et al, 1989) and hypophysectomized rats (Buhl et al, 1982), indicate that the suppression of testosterone following the administration of GnRH analogs may not be the only cause for the disruption of spermatogenesis.

We have recently noted that the synergistic effects of follicle-stimulating hormone (FSH) and testosterone on the maintenance of complete spermatogenesis in hypophysectomized rats correlated with the transport of androgen binding protein (ABP) toward the epididymis (Huang et al, 1991). These results suggest that the ABP distribution in the testis may play a critical role in the final stages of spermiogenesis. In order to investigate similarities between hypophysectomy and selective gonadotropin suppression, the present study examined the correlation of the qualitative completion of spermiogenesis and the distribution of ABP

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in the testis, epididymis, and serum subsequent to the administration of a potent GnRH-antagonist (GnRH-A, Ac-D[2] Nal[1], 4Cl-D Phe[2], D-Trp[3], D-Arg[6], D-Ala[10]).

Materials and Methods

Experiment 1

Mature, male Sprague-Dawley rats (weighing 300 g to 350 g) were given daily subcutaneous injections of 0, 10, 50, 100, or 500 μg GnRH-A per kilogram of body weight in 0.1 ml phosphate buffered saline for 2 or 4 weeks. Animals were caged in groups of three in an air conditioned, light controlled animal room, and were fed Purina rat chow and water ad libitum.

Retro-orbital sinus blood samples (3 ml to 4 ml) were collected from each animal under ether anesthesia 24 hours after the last GnRH injection. Animals were subsequently killed by overdose of ether.

One-half of one testis from each animal was fixed in Bouin's solution and processed for histologic examination. The sections were stained with periodic acid-Schiff reagent and counterstained with hematoxylin (Preece, 1972). The stages of the seminiferous epithelial cycle were determined according to the development of acrosome (Leblond and Clermont, 1952). The remaining testicular tissue and epididymis were stored at −70°C for subsequent testosterone and ABP measurement.

Experiment 2

In order to investigate whether the alterations in serum concentration or testicular and epididymal ABP content noted in the previous experiment were associated with alterations in the bidirectional secretion of ABP, mature Sprague-Dawley male rats weighing 250 g to 300 g were given daily injections of 0, 25, or 250 μg GnRH-A per kilogram of body weight in 0.1 ml phosphate buffered saline for 2 weeks. Retro-orbital sinus blood samples were collected from each animal 24 hours after the last injection and before they were killed as described above. Testes from each animal were used immediately for the collection of interstitial fluid (ITF) and seminiferous tubular fluid (STF) by the method described by Turner et al (1984). The epididymes were stored at −70°C for subsequent ABP assay.

Hormone Measurement

Serum concentrations of FSH and luteinizing hormone (LH) were determined in 0.1 ml of serum by double antibody radioimmunoassay as described previously (Huang and Hembree, 1979). Reagents provided by the National Institute of Arthritis, Diabetes, Digestive and Kidney Disease (NIADDK); NIADDK rat FSH RP-2, rat FSH I-5, anti- rat FSH S-11, and rat LH PR-2, rat LH I-6, and anti-rat LH S-7; were used for FSH and LH assay, respectively. The sensitivity of the assay (95% binding) was 0.15 ng/ml and 2.6 ng/ml for LH and FSH, respectively. The inter- and intra-assay coefficient of variation was approximately 12% and 8%, respectively, for both assays. To avoid interassay variation, all samples from experiment 2 were measured for LH or FSH in one assay.

Serum and testicular testosterone concentrations were determined by radioimmunoassay in ether extracts without chromatography (Huang et al, 1991) using antiserum provided by ICN Immunobiological (Lisle, IL). The sensitivity of this assay was 2 pg/tube, and the intra- and inter-assay coefficients of variation were 5% and 12%, respectively.

Androgen Binding Protein Measurement

Decapsulated testicular tissues and whole epididymis were homogenized with a polytron homogenizer for 5 to 10 seconds in ice cold buffer containing 20 mmol/L Tris, 50 mmol/L $CaCl_2$, and 10% glycerol (pH 7.4). The tissue to buffer ratios were 1:2 for testis and 1:9 for epididymis. The homogenates were centrifuged at 40,000g for 1 hour at 4°C. The cytosols were collected and stored at −30°C until assay.

Androgen binding protein in serum, ITF, STF, and tissue cytosols was measured by radioimmunoassay (Gunsalus et al, 1978) using the reagents provided by NIADDK. The sensitivity was approximately 30 ng/tube with inter- and intra-assay coefficients of variation of 11% and 6%, respectively. To avoid inter-assay variation, samples from each experiment were measured in a single assay.

Statistics

Analysis of variance was employed to detect the significance of the treatment effect among groups. When the treatment effect was significant, Scheffe's multiple range test was used to identify the difference between groups.

Results

Experiment 1

Daily administration of GnRH-A to adult male rats for 2 or 4 weeks resulted in dose dependent decreases in both testicular ($P < 0.05$) and epididymal weights ($P < 0.01$; Table 1).

Responses of testicular testosterone to the GnRH-A treatment varied according to the doses used. At the end of either a 2-week or 4-week injection period, testicular testosterone concentrations in rats receiving 10 μg of GnRH-A per kilogram of body weight were 70% and 140%, respectively, above control levels (Fig 1). The increase at the end of the 4-week treatment period was statistically significant ($P < 0.05$). Testicular testosterone concentration was unaltered in rats given 50 μg per kilogram of body weight, but was suppressed significantly ($P < 0.01$) in rats given two higher doses of GnRH-A. Changes in serum testosterone levels followed the same pattern as those noted in testicular testosterone (Table 1).

After 2 weeks of GnRH-A treatment, testicular ABP content was increased by 15% to 38% ($P < 0.05$ for the 10 μg/kg group and 500 μg/kg group; Fig 2). At the end of 4 weeks, while testicular ABP content remained normal among rats receiving 10 μg of GnRH-A per kilogram of body weight, it tended to decrease in a dose-dependent

Table 1. *Effects of gonadotropin releasing hormone antigen (GnRH-A) upon organ weight and serum testosterone concentration*

GnRH-A dose (μg/kg of body weight)	No.	Duration of treatment (wks)	Organ weights (mg)		Serum testosterone (ng/ml)
			Testis	Epididymis	
Control	6	2	1935 ± 62	645 ± 21	2.32 ± 0.54
	6	4	1962 ± 71	690 ± 23	2.72 ± 0.81
10	6	2	1833 ± 66	576 ± 18	3.53 ± 0.50
	6	4	1834 ± 50	606 ± 11	4.53 ± 1.67
50	6	2	1671 ± 66*	562 ± 17*	2.62 ± 0.58
	6	4	1543 ± 57*	498 ± 29*	1.88 ± 0.65
100	6	2	1531 ± 60*	394 ± 41†	0.18 ± 0.11†
	6	4	930 ± 144†	249 ± 27†	0.63 ± 0.16†
500	6	2	1126 ± 53†	263 ± 7†	0.17 ± 0.04†
	6	4	517 ± 22†	185 ± 7†	0.37 ± 0.05†

Results are expressed as the mean ± SEM.
* $P < 0.05$ versus control.
† $P < 0.01$ versus control.

manner in those receiving higher doses of GnRH-A ($P < 0.01$).

After 2 weeks, epididymal ABP content remained unaffected among rats given daily doses of 10 μg or 50 μg of GnRH-A per kilogram of body weight, but was suppressed significantly in rats given the two higher doses ($P < 0.01$). It was suppressed even further by the end of the 4th week of GnRH-A treatment ($P < 0.05$, Fig 3). Serum ABP concentrations were significantly increased in comparison to control levels after 2 weeks of daily injection of GnRH-A ($P < 0.01$, Fig 4). At the end of the fourth week, however, the elevated serum ABP levels persisted only in rats treated with 100 μg of GnRH-A per kilogram of body weight.

In control animals, the presence of step 18–19 spermatids at the luminal edge of stages VII through VIII of the cycle of the seminiferous epithelium demonstrates the completion of spermatogenesis (Fig 5). In rats given the two lower doses of GnRH-A, maintenance of qualitatively complete spermatogenesis was demonstrated by the presence of step 18–19 spermatids, but sloughing of early germ cells was also noted (Fig 6). While spermatogenesis was qualitatively normal at least up to step 8 of spermiogenesis, the absence of elongated spermatids in the animals given the two higher doses of GnRH-A demonstrates that the second half of spermiogenesis was impaired in these animals (Fig 7). The incomplete spermatogenesis was confirmed by the absence of spermatozoa in the epididymis (data not shown).

Experiment 2

Figure 8 shows the serum LH, FSH, and testosterone responses to daily injections of GnRH-A for 2 weeks. While both LH and FSH were suppressed significantly in a dose-dependent manner ($P < 0.01$), a significant suppression of serum testosterone was noted only in those rats given 250 μg of GnRH-A per kilogram of body weight ($P < 0.01$).

Table 2 shows the effects of GnRH-A administration upon the distribution of ABP in different testicular compartments. While administration of 25 μg of GnRH-A per kilogram of body weight did not alter ABP levels in the testis or epididymis, serum ABP levels were significantly

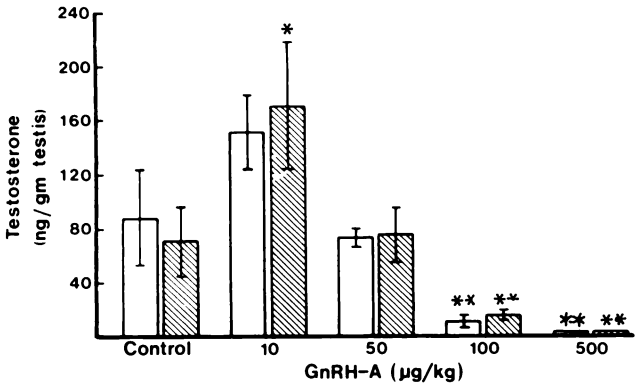

FIG. 1. Testicular testosterone concentrations (mean ± SEM ng/gm) of rats after daily injection of various doses of GnRH-A for 2 weeks (open bar) or 4 weeks (hatched bar). *$P < 0.05$ versus control; **$P < 0.01$ versus control; N = 6.

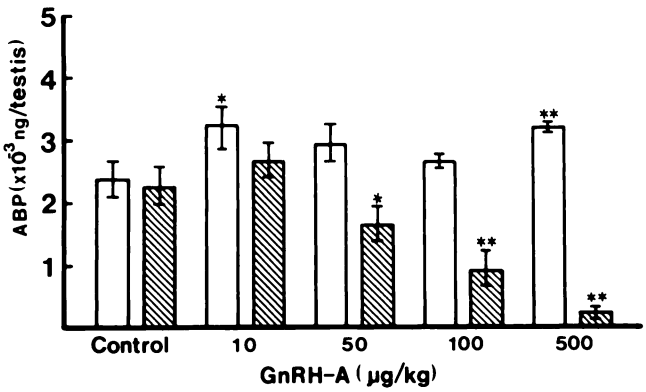

FIG. 2. Testicular androgen binding protein content of adult rats receiving daily injections of various doses of GnRH-A for 2 weeks (open bar) or 4 weeks (hatched bar). Results are expressed as mean ± SEM ng/testis. **$P < 0.01$ versus control; N = 6.

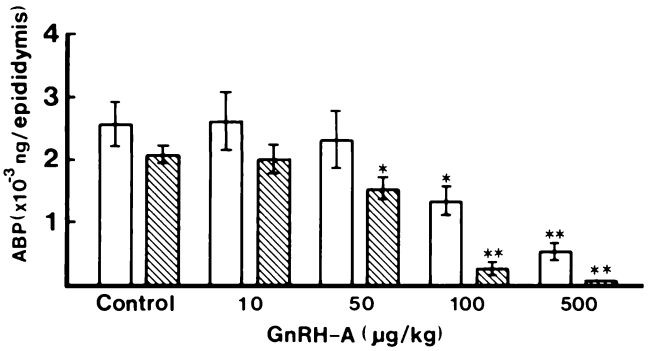

FIG. 3. Epididymal androgen binding protein content (mean ± SEM ng/epididymis) of adult rats given various doses of GnRH-A for 2 weeks (open bar) or 4 weeks (hatched bar). *$P < 0.05$ versus control; **$P < 0.01$ versus control; N = 6.

elevated ($P < 0.05$). In contrast, injection of 250 μg/kg of GnRH-A resulted in a significant increase in ABP concentration in both ITF and serum. While ABP concentration in STF also increased, the change did not reach statistical significance. Moreover, ABP content of the epididymis was significantly decreased ($P < 0.05$). Spermatogenesis was complete in the group given 25 μg of GnRH-A per kilogram of body weight, but was arrested at early spermiogenesis in the group given 250 μg (data not shown).

Discussion

Previously, Huang and Boccabella (1988) reported that the maintenance of spermatogenesis in intact animals given various doses of exogenous testosterone corresponded to biphasic changes in the testicular ABP content. Recently, we reported that the FSH enhancement of the qualitative and quantitative maintenance of spermatogenesis in hypophysectomized animals given testosterone was related to an

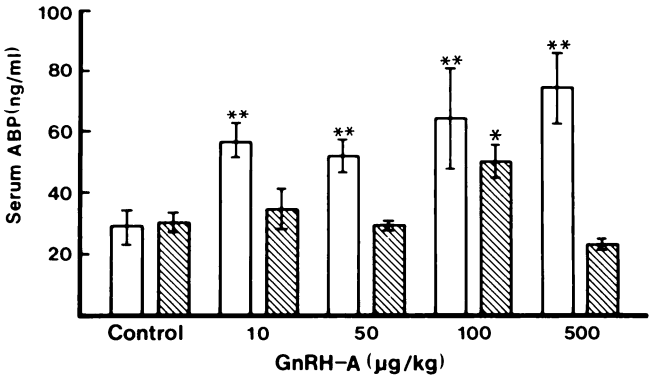

FIG. 4. Serum androgen binding protein concentrations (mean ± SEM ng/ml) of adult rats after 2 weeks (open bar) or 4 weeks (hatched bar) of injection with various doses of GnRH-A. *$P < 0.05$ versus control; **$P < 0.01$ versus control; N = 6.

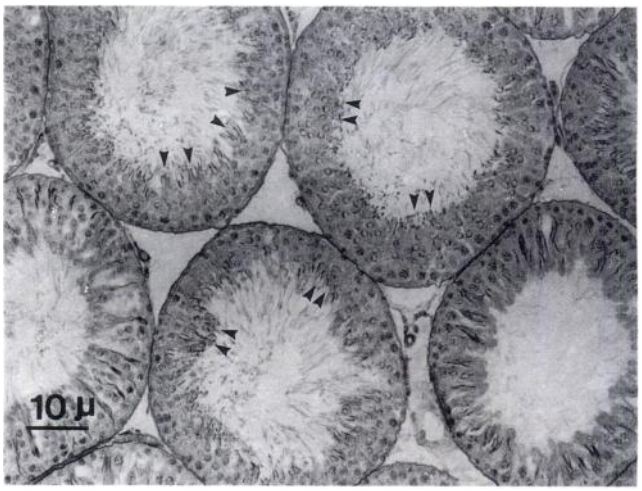

FIG. 5. Testicular histology of a control rat. The presence of step 18–19 spermatids (arrowheads) at the luminal edge of stage VII–VIII of the cycle of the seminiferous epithelium demonstrates the completeness of spermatogenesis (magnification = ×150).

increase in the transport of ABP to the epididymis (Huang et al, 1991). These observations suggest that Sertoli cell ABP production and distribution may have a specific role in the regulation of spermatogenesis.

The dose-dependent suppression of the pituitary–testicular hormonal axis by GnRH antagonists is analogous to that seen among hypophysectomized animals given various regimens of hormonal replacement. If ABP has a functional significance in spermiogenesis, the disruption of spermatogenesis following administration of GnRH-A should be associated with an alteration of ABP distribution

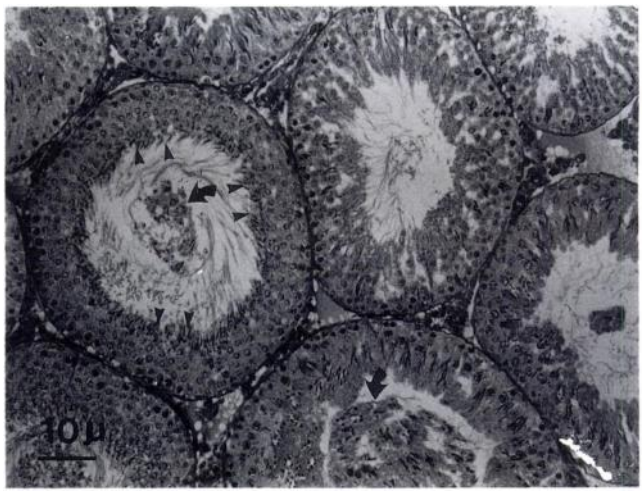

FIG. 6. Testicular histology of a rat given 50 μg/kg of GnRH-A for 4 weeks. Note the presence of step 18–19 spermatids (arrowheads) at the luminal edge of the stage VII–VIII of the cycle of the seminiferous epithelium, and the sloughing of the early germ cells in the center of the lumen (arrows; magnification = ×150).

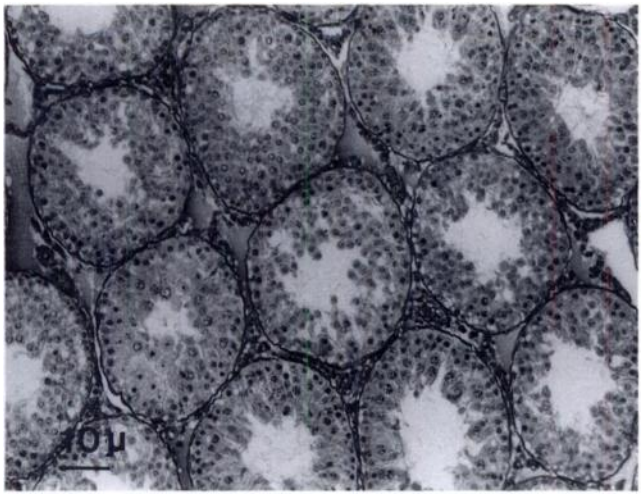

FIG. 7. Testicular histology of a rat given 100 μg/kg of GnRH-A for 2 weeks. Note absence of elongated spermatids in all tubules (magnification = ×150).

in the testis and epididymis similar to that observed among hypophysectomized rats. The present study examined this hypothesis.

After 2 weeks of daily injections of high doses of GnRH-A, spermatogenesis was arrested at the early stage of spermiogenesis. This was associated with a decrease in epididymal ABP content while testicular ABP content was either normal or increased. In contrast, qualitatively normal spermatogenesis was maintained in rats given lower doses of GnRH-A, and epididymal ABP content in these animals was normal. The relationship between the status of spermiogenesis and the ABP distribution in testis and epididymis was consistent with our findings in hypophysectomized rats administered testosterone and FSH (Huang et al, 1991).

While the suppression of testosterone in rats given high doses of GnRH-A is expected, the increase in testicular and serum testosterone levels among rats given 10 μg of GnRH-A per kilogram of body weight was suprising. These results cannot be accounted for by changes in serum LH levels, and a significant stimulation of Leydig cells by GnRH-A is unlikely (Huhtaniemi et al, 1987). In light of the recent findings that Leydig cell function can be modulated by Sertoli cells and germ cells (reviewed by Skinner, 1991), the higher testosterone levels noted among rats given 10 μg of GnRH-A per kilogram of body weight may reflect an adaptive response of Leydig cells to the changes in the microenvironment within the seminiferous tubules, even though the changes were not severe enough to cause a significant germ cell loss.

A decrease in testicular and epididymal levels of ABP after 4 weeks of high doses of GnRH-A is consistent with the decrease in its production attributable to the decrease in testicular testosterone (Sharpe and Bartlett, 1987). The maintenance or increase in testicular ABP content, however, after 2 weeks of administration of 100 μg to 500 μg of GnRH-A per kilogram of body weight was also associated with a marked decrease in testicular testosterone concentration. Nonetheless, since serum FSH levels were significantly decreased in rats given 25 μg of GnRH-A per kilogram of body weight, the increased testicular ABP content seen at 2 weeks is unlikely to have resulted from an increase in ABP synthesis.

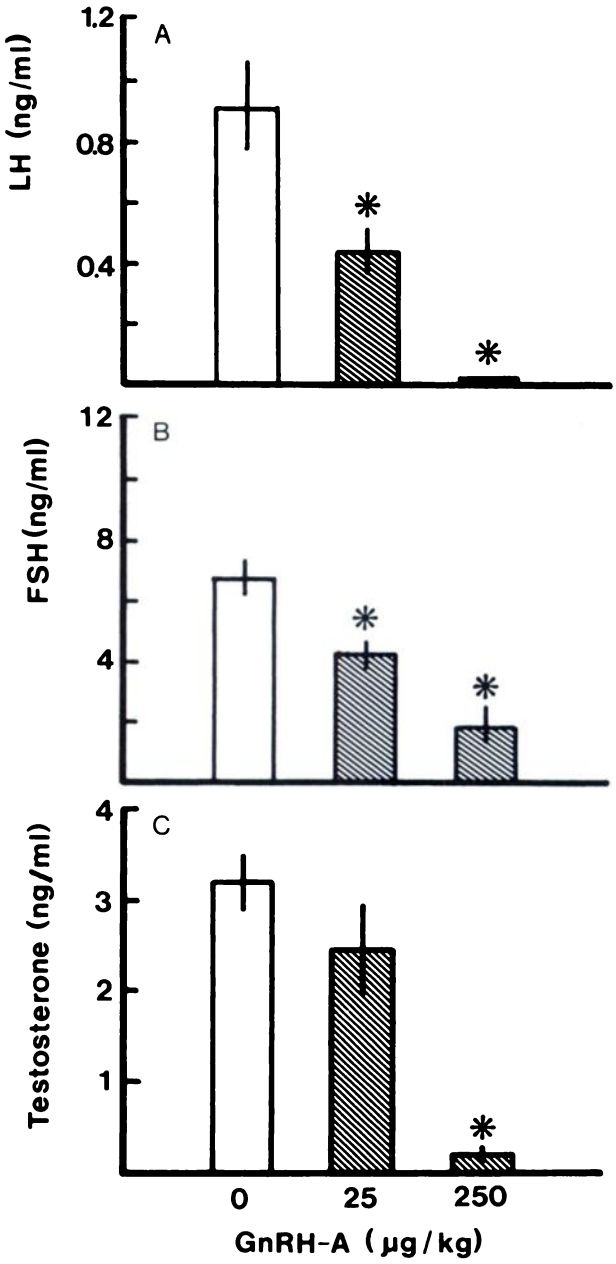

FIG. 8. Serum luteinizing hormone (A), follicle stimulating hormone (B), and testosterone (C) responses to daily injections of 25 μg/kg or 250 μg/kg GnRH-A for 2 weeks. *$P < 0.01$ versus control; N = 5.

Among hypophysectomized rats, although an increase in

Table 2. *Effects of GnRH-A on the distribution of androgen binding protein in testis, epididymis, and serum*

GnRH-A dose (μg/kg of body weight)	N	Testis (μg/testis)	Epididymis (μg/epididymis)	STF (μg/ml)	ITF (μg/ml)	Serum (ng/ml)
control	5	1.89 ± 0.20	6.79 ± 1.10	4.59 ± 0.57	5.49 ± 0.50	86 ± 5
25	5	2.25 ± 0.22	4.89 ± 0.74	5.04 ± 0.77	6.92 ± 0.59	101 ± 6*
250	5	2.13 ± 0.50	1.29 ± 0.40†	6.51 ± 0.28	8.48 ± 0.43*	146 ± 9*

STF = seminiferous tubule fluid; ITF = interstitial fluid.
All values are presented as the mean ± SE.
* $P < 0.05$.
† $P < 0.01$.

testicular ABP content was noted after testosterone replacement, an increase in epididymal ABP content was observed only in rats who were given daily FSH injections (Huang et al, 1991). This result is consistent with previous findings that FSH is essential for the transport of ABP toward the epididymis in the adult hypophysectomized rat (Gunsalus et al, 1980). While the mechanism for this phenomenon remains to be defined, FSH may promote canalization of the seminiferous tubules, whereas testosterone may facilitate transport (Danzo et al, 1990). Since there was no decrease in ABP concentration in the STF among animals given 250 μg of GnRH-A per kilogram of body weight for 2 weeks, the marked suppression of epididymal ABP content among rats given 100 μg or more GnRH-A per kilogram of body weight may result from a failure in the transport of testicular fluid into the epididymis due to a decrease in serum FSH levels, decreased testicular testosterone, or both (Jegou et al, 1983). Thus, the increased testicular ABP levels noted at 2 weeks may represent an accumulation of ABP within the testis.

The elevations of ABP levels in serum and ITF among rats given higher doses of GnRH-A for 2 weeks are consistent with the alterations of the bidirectional release of ABP among animals in whom spermatogenesis was disrupted under various experimental conditions, such as progestin (Lobl et al, 1983), ethan dimethane sulphonate (Sharpe and Bartlett, 1987; Morris et al, 1988), local heating of the testis, or artificially induced cryptorchidism (Sharpe and Bartlet, 1987). This is demonstrated by the presence of normal or elevated serum ABP after 4 weeks of administration of high doses of GnRH-A, despite markedly suppressed testicular ABP content.

It is of interest that serum ABP was also elevated in rats given 10 μg of GnRH-A per kilogram of body weight, in whom spermatogenesis had been qualitatively maintained. Whereas this finding suggests that alterations in the bidirectional release of ABP may reflect subtle changes in the seminiferous epithelium, there is no consistent relationship between the ABP release and the normalcy of spermiogenesis. In this regard, serum ABP was highly elevated among adult hypophysectomized rats, and was elevated even further during the FSH and testosterone induced completion of spermiogenesis (Huang et al, 1991). Future studies of the hormonal regulation of the post-translational modification of ABP and its distribution in the seminiferous epithelium are warranted so that a better understanding of the functional significance of ABP in the regulation of spermatogenesis can be developed.

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Seventh European Workshop on Molecular and Cellular Endocrinology of the Testis

Castle Elmau, Bavarian Alps, Germany—May 5–10, 1992

The plenary sessions will include: endocrine and paracrine regulation of testicular function; inhibin, activin, and growth factors; gonadotropin receptors; androgen receptor, and testosterone metabolism; spatial arrangement of germ cells; genetic control of spermatogenesis; and sperm maturation in the epididymis.

Invited speakers include: E. Adashi (USA), A. Brinkman (NL), B. Cooke (UK), T. Cooper (D), G. Jegou (F), L. Martini (I), J. Mather (USA), M. ven Noort (NL), W. Schulze (D), P. Vogt (D), D. Wolgemuth (USA), and P. Wong (Hong Kong).

Information: Dr. E. Nieschlag, Institute of Reproductive Medicine, University of Münster, Steinfurter Straße 107 D-4400 Münster, Germany. Tel: 49-251-836097; Fax: 49-251-836093.

First International Symposium on Neurobiology and Neuroendocrinology of Aging

Bregenz, Austria—July 19–24, 1992

Topics to be discussed include changes in neurotransmission related to aging and Alzheimer Disease, hypothalamic mechanisms of reproductive aging, and changes in growth hormone release during aging. The program will consist of invited lectures, poster presentations, and informal discussions. The symposium is sponsored by Voralberger Landesregierung and Southern Illinois University.

Information:

Dr. Andrezej Bartke, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL 62901-6512; or
Dr. Ezio Giacobini, Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62794-9230.