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Final Report

USAID Grant DAN-5053-G-SS-7025-00

Direct Gonadal Effects of GnRH-Analogues on the Primate Testis

David R. Mann Ph.D.
Principal Investigator
Department of Physiology
Morehouse School of Medicine

This is the final progress report of USAID grant (DAN-5053-G-SS-7025-00) funded for the period from July 1, 1987 - December 31, 1988. During the funding period, we published four manuscripts and five abstracts that resulted primarily from work supported by this grant.

The work supported by this project also contributed to the preliminary data which resulted in the funding of a major grant on osteoporosis by Genentech, Inc. I have provided copies of published manuscripts and abstracts which detail our results on the project.

I would like to thank the USAID for their confidence and support of our research program over the last several years.

Sincerely,

David R. Mann

David R. Mann, Ph.D.
Department of Physiology

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EFFECT OF ANDROGENS ON SERUM LIPOPROTEINS IN MALE RATS.

Rosa L. King, Milton F. Bryant and David R. Mann; Morehouse School of Medicine, Atlanta, GA.

Androgens used to treat osteoporosis and hypogonadism, and to stimulate muscular development in athletes may increase the risk of cardiovascular disease. The degree of the risk may be related to the type of androgen administered (free versus esterified) and the route of administration, although to date such a study has not been undertaken. In the present study, we compared the effect of testosterone (T) and testosterone enanthate (TE) on serum total cholesterol (C), HDL-C, LDL/VLDL-C and triglycerides (TG) in the castrate rat model. Adult male rats were either sham-castrated or castrated. Castrates received biweekly sc injections of either oil, 10 or 50 mg/kg BW of TE or no treatment (empty silastic capsule), 1 T capsule (2 cm) or 4 T capsules. After 5 wk of treatment, rats that received the lower dose of T or TE had serum T levels in the high normal range, those animals treated with the higher dose of T or TE had serum T values that were 5-10 fold higher than intact control levels. Castration increased serum C and HDL-C, but had no effect on serum LDL/VLDL-C or TG. Both T and TE treatment reduced serum C and HDL-C in castrates (TE was more effective), but only TE also reduced serum LDL/VLDL-C values. T and TE had equivocal effects on serum TG. The data suggest that the type of androgen administered and/or route of administration may be critical in determining the effect on circulating lipoproteins and ultimately the cardiovascular risk of the therapy. Supported by MB-01-MSM and DAN-5053-G-SS-5081-00.

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EXAMPLE

DELAYED AMPLIFICATION OF CYTOMEGALOVIRUS INFECTION IN THE PLACENTA DURING LATE GESTATION.

Elizabeth Goff, Brigitte P. Griffith, and John Booss; University of Connecticut School of Medicine, Farmington, CT.

Cytomegalovirus (CMV), the most common viral infection in the human fetus, can result in severe generalized illness.....

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BIOACTIVE AND IMMUNOACTIVE LH AND THE COMMON ALPHA SUBUNIT DURING GnRH AGONIST TREATMENT IN FEMALE RHESUS MONKEYS.

D.C. Collins, K.G. Gould* and D.R. Mann, VA Medical Center, Morehouse School of Medicine, Emory University School of Medicine and Yerkes Primate Research Center, Atlanta, GA.

The mechanism by which GnRH agonists suppress gonadal function in primates has not been fully defined. GnRH agonist administration to humans decreases the ratio of bioassayable to immunoassayable (B/I) LH, perhaps by secretion of less biologically potent species of LH. In this study, we confirm a decrease in the B/I LH ratio in another species (rhesus monkey). Female monkeys (N=5) were treated for 3 mo by continuous infusion (osmotic minipump) with 25 µg/day of a GnRH agonist (Wyeth Laboratories, WY-40972). Blood samples were taken over a control cycles and at weekly intervals during the first 8 wk of the treatment and posttreatment periods for measurement of B-LH, I-LH and the common alpha subunit. During a pretreatment menstrual cycle, I-LH ± SE ranged from 7.4 ± 1.6 ng/ml (NICHD-rhLH) to an ovulatory peak of 110 ± 39 ng/ml; corresponding pretreatment values for B-LH were 9.4 ± 2 and 60 ± 7 ng/ml; for the alpha subunit, 32 ± 5 and 135 ± 30 ng/ml, respectively. By 8 wk of GnRH agonist treatment, the values declined to 6.1 ± 1.0 ng/ml for I-LH, 1.4 ± 0.4 ng/ml for B-LH and 14.4 ± 6 ng/ml for the alpha subunit. The B/I LH ratio, which varied between 0.76 ± 0.26 and 1.92 ± 0.52 during the pretreatment menstrual cycle. declined to 0.21 ± 0.02 at 8 wk of treatment, reflecting a quantitatively greater decrease in B-LH compared to I-LH. These data suggest that agonist treatment leads to secretion of quantitatively different LH in the female monkey. Supported by USAID grant DAN-5053-G-55-5081-00, VA Project #1517-001, and NIH Grant #RR-00165.

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COMBINED GnRH AGONIST AND TESTOSTERONE TREATMENT INDUCES AZOOSPERMIA IN MALE MONKEYS. D.R. Mann, D.C. Collins, T. Duffey, T. Buckner and K.G. Gould, Morehouse Sch. of Med., Emory Univ. Sch. of Med./V.A. Med. Ctr. and Yerkes Primate Ctr., Atlanta, GA 30310.

Five adult rhesus monkeys are being treated continuously with 25 ug/day of a gonadotropin releasing hormone agonist (Ag) using minipumps. Five other monkeys are receiving the Ag plus testosterone (T) therapy. Serum LH and T rose initially with the onset of Ag treatment, but then fell precipitously to basal values. In monkeys being treated with only Ag, serum LH continued to fall to minimum levels of detection (0.20 ug/ml) and serum T decreased to castrate levels (0.5 ng/ml). In monkeys that are receiving T replacement, serum T values stabilized at physiological levels (4-5 ng/ml). Sperm counts declined from pretreatment values of 1.9×10^9 to azoospermia by 12 wk Ag treatment. T therapy did not interfere with the induction of azoospermia. These data suggest that in contrast to a previous report (Akhtar et al., Int. J. Androl., 6:461, 1983), T therapy does not interfere with Ag-induced azoospermia in monkeys. Thus, it may be possible to utilize Ag's as a male contraception even if it is necessary to provide androgen therapy to maintain libido. (Supported by AID Grant #DAN-5053-G-SS-5081-00).

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FAILURE OF IN VIVO AND IN VITRO GONADOTROPIN RELEASING HORMONE AGONIST TREATMENT TO DIRECTLY ALTER STEROIDOGENESIS BY THE PRIMATE TESTIS. D.R. Mann, K.G. Gould*, S.R. Adams*, and D.C. Collins, Morehouse School of Medicine, Yerkes Primate Research Center, Centers for Disease Control and Emory Univ./VA Medical Center, Atlanta, GA 30310-1495.

Three experiments were performed to determine whether GnRH agonists directly affect testicular steroidogenesis in primates. In Experiment 1, adult male monkeys were treated for 10 mo with a GnRH agonist (Ag; 25 µg/day; Wy-40972; Wyeth Labs) alone or in combination with testosterone (T) administration to maintain serum T levels between 4-5 ng/ml. The response to HCG (50 IU; im) was determined before and at the end of treatment. In Experiment 2, 100 ng of Ag or saline was infused over 1 min into the testicular artery (N = 7) and blood samples collected from the testicular and femoral veins over the next 2 h for LH and T assay. In Experiment 3, testicular interstitial cells from a peripubertal monkey were incubated 24 h with 10^{-9} to 10^{-5} M Ag. The effect of Ag on basal and HCG-stimulated T production from these interstitial cells was determined. In Experiment 1, basal and peak values of serum T after HCG stimulation were reduced by 10 mo of Ag administration (pretreatment basal and HCG-stimulated T = 5.2 ± 0.8 and 27.0 ± 1.8 ng/ml vs treatment basal and HCG-stimulated T = <0.7 and 5.9 ± 2.8 ng/ml), but the relative response (ratio of the peak serum T after HCG to the basal value) of the testis to HCG was not altered by Ag treatment alone. In contrast, combined Ag and T treatment reduced the relative response of the testis to HCG to 28% of the pretreatment level. In Experiment 2, acute infusion of 100 ng of Ag into the testicular artery of adult male monkeys did not alter systemic serum LH or T or serum T in the testicular vein over the next 2 h. In Experiment 3, Ag treatment for 24 h failed to alter basal or HCG-stimulated T production from isolated testicular interstitial cells. These data clearly demonstrate that Ag treatment does not directly alter steroidogenesis by the primate testis. However, chronic administration of T greatly attenuated the relative response to HCG in Ag-treated adult male monkeys. The mechanism of this reduced response to HCG remains to be determined.
(Supported by USAID Grant #DAN-5053-G-SS-5081-00.)

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Effect of continuous infusion of a low dose of GnRH antagonist on serum LH and testosterone concentrations, spermatogenesis and semen quality in the rhesus monkey (*Macaca mulatta*)

D. R. Mann, D. C. Collins†, M. M. Smith* and K. G. Gould‡

*Departments of Physiology and *Pathology, Morehouse School of Medicine, Atlanta, GA 30310; †Medical Research Service, VA Medical Center and Departments of Medicine and Biochemistry, Emory University School of Medicine, Atlanta, GA 30033; and ‡Yerkes Regional Primate Research Center, Atlanta, GA 30322, U.S.A.*

Summary. Treatment of 4 adult male rhesus monkeys for 8–12 months with 100–400 μg of a GnRH antagonist/day by means of using osmotic minipumps led to suppressed serum concentrations of LH and testosterone followed by various degrees of recovery toward pretreatment values. The serum LH response to a challenge of native GnRH was reduced by 30–75% during antagonist treatment. The serum testosterone response to GnRH was exaggerated above the response in the pretreatment period, suggesting hypersensitivity of the testis to gonadotrophin. Antagonist administration under these conditions did not alter body weight or abolish ejaculatory response. Antagonist infusion caused a 96% decrease in sperm counts. Spermatozoa recovered during the final month of antagonist treatment showed a reduced ability to penetrate denuded hamster ova. Testicular biopsies performed at the end of antagonist treatment revealed persistent spermatogenesis. However, the cellularity of the seminiferous tubules was decreased below that of pretreatment biopsies. The results of this study suggest that the amount of testosterone needed to maintain normal spermatogenesis is greater than that needed to maintain electroejaculatory response in monkeys.

Introduction

The development of gonadotrophin-releasing hormone (GnRH) antagonists has led to a number of attempts to use these analogues to induce azoospermia. However, administration of large doses has led to undesirable side effects. Daily injection or continuous infusion of milligram doses of GnRH antagonists for 9 weeks induced azoospermia in male monkeys, but was associated with a significant loss of body weight (Weinbauer *et al.*, 1984; Akhtar *et al.*, 1985). Subcutaneous administration of large doses of the antagonist, Ac-(2)D-NAL^1-pF-D-Phe^2-D-Trp^3-D-Arg^6-GnRH, caused facial oedema and oedema of the extremities in rats and reduced spontaneous activity in monkeys (Schmidt *et al.*, 1984). These results suggested that it might be impossible to attain reliable azoospermia without significant side effects, thus reducing the possibility of using GnRH antagonists for contraception.

However, the provision of low levels of GnRH agonist by continuous infusion is an effective way of suppressing LH and testosterone secretion and the response to a GnRH challenge (Akhtar *et al.*, 1983; Mann *et al.*, 1984). In this study, we examine the effect of continuous infusion of a GnRH antagonist via osmotic minipump on basal LH and testosterone secretion and the response to an i.v. bolus of GnRH in male rhesus monkeys. In addition, we investigated the effect of low-dose continuous infusion of GnRH antagonist treatment on sperm count and quality.

6

Materials and Methods

Four adult male rhesus monkeys were infused continuously with a GnRH antagonist (Ac-(2)D-NAL[1]-pF-D-Phe[2]-D-Trp[3]-D-Arg[6]-GnRH) for 36 or 50 weeks using a s.c. implanted (intrascapular area) osmotic minipump (Alza Corporation, Palo Alto, CA; model 2 ml-4). Two monkeys (A and B) were treated with 100 μg/day for 10 weeks, then 200 μg/day for 20 weeks and finally 400 μg/day for an additional 20 weeks. The other 2 animals (C and D) were treated, with the same antagonist, with 200 μg/day for 16 weeks and then 400 μg/day for 20 weeks. New pumps were implanted every 4 weeks. Animals were maintained in a temperature- (23°C) and light- (12 h light:12 h dark) controlled room throughout the study. Blood samples (4 ml) were taken by venepuncture from the saphenous vein of restrained conscious animals during the pretreatment period (3–5 samples), at 3 and 7 days of treatment and thereafer at weekly intervals. Serum samples were stored frozen at −20°C until assayed for LH and testosterone.

At monthly intervals throughout the study, all animals were injected with an i.v. bolus of 25 μg synthetic GnRH (United States Biochemical Corporation, Cleveland, OH) to test the serum LH and testosterone response. Blood samples were collected 15 min before administration, just before administration and at 15, 30, 60 and 120 min after GnRH injection. Serum samples were stored at −20°C until LH and testosterone were measured.

Serum concentrations of LH were determined using the mouse interstitial cell bioassay (Van Damme *et al.*, 1974) with the NIADDK rhesus monkey pituitary gonadotrophin standard (LER 1909-2) as the reference preparation. The intra- and interassay coefficients of variation were 14·1 and 14·7% respectively. The minimal detectable dose for LH was 0·2 μg/ml. Serum concentrations of testosterone were determined by radioimmunoassay as previously described (Perachio *et al.*, 1977). The intra- and interassay coefficients of variation for testosterone were 5·1 and 6·8%. The minimal level of detection for the testosterone assay was 0·5 ng/ml.

Testicular measurements were made at monthly intervals with Lange calipers with a compression pressure of 15 g as previously described (Mann *et al.*, 1985). The formula used for testicular volume was $4/3\ a^2b$ (a = short axis; b = long axis). Testicular needle biopsies were performed on all animals during the pretreatment period, at the end of antagonist treatment and at 16 weeks of recovery. Tissue was processed for routine histological examination as previously described (Mann *et al.*, 1985). Biopsy samples were embedded in paraffin wax, cut at 4 μm and stained with haematoxylin and eosin or with Masson's trichrome for connective tissue. The mean ± s.e.m. of the number of seminiferous tubules collected from biopsies was 34·6 ± 4·7 (range 10–47).

Animals were subjected to rectal probe ejaculation at approximately 4-week intervals as reported previously (Gould *et al.*, 1978) to determine sperm counts, percentage live spermatozoa and percentage motile spermatozoa. Sperm samples from all treated monkeys, and from 4 untreated, age-matched animals housed with the treated monkeys, were tested for their ability to penetrate denuded hamster oocytes during the last month of antagonist treatment and again 16 and 22 weeks after the end of antagonist treatment. The hamster ova penetration test was performed essentially as described by Yanagimachi *et al.* (1976). Hamster oocytes were recovered from superovulated hamsters (25 i.u. PMSG i.p. followed by 50 i.u. hCG i.p. 3 days later). The cumulus cells were removed by incubating in 1% hyaluronidase for 15 min and the zonae were dissolved by transferring the oocytes to a 0·1% trypsin solution in Dulbecco's medium for 10 min. After dissolution of the zonae pellucidae, the denuded oocytes were transferred to Ham's F10 medium containing 10% human cord serum. After washing, 10 oocytes were cultured in 0·5 ml culture medium in each well of an 8-well culture dish (Lab Tek, Inc., Naperville, IL).

Semen samples were washed twice in culture medium, concentrated by centrifugation and further washed by allowing motile spermatozoa to 'swim up' into Ham's culture medium which had been overlaid on the sperm pellet. The 'swim-up' procedure took 30–60 min. Washed spermatozoa were added to the denuded oocytes at a ratio of 25 000 motile spermatozoa/oocyte, and 10–30 oocytes were used per assay. Incubations were performed at 37°C. At 18 h the oocytes were compressed under a cover slip and stained with aceto-orcein or lacmoid. The assay was scored according to the percentage of oocytes penetrated per well. After penetration, the sperm head undergoes swelling and decondensation, and can be identified by using phase-contrast microscopy.

The statistical significance of all results, except those from the hamster ova penetration assay, was assessed by analysis of variance and the least significant difference for multiple comparisons as needed (Keppel, 1973). The results from the hamster ova penetration assay were tested for statistical significance by χ^2 test.

Results

The effects of GnRH antagonist infusion on serum LH and testosterone for Monkeys A and B are shown in Fig. 1. Administration of 100 μg antagonist/day induced an immediate fall in serum LH values in Monkey A, and these remained suppressed throughout the period of treatment. In this animal, serum testosterone concentrations decreased initially, but then recovered to pretreatment values before declining again when the dose of antagonist was increased to 200 μg/day, and remaining below pretreatment values until treatment was terminated. Monkey B was not as responsive to the antagonist; there was no consistent suppression of serum LH or testosterone concentrations even after the dose was increased to 400 μg/day. Instead, there were alternate periods of suppression and

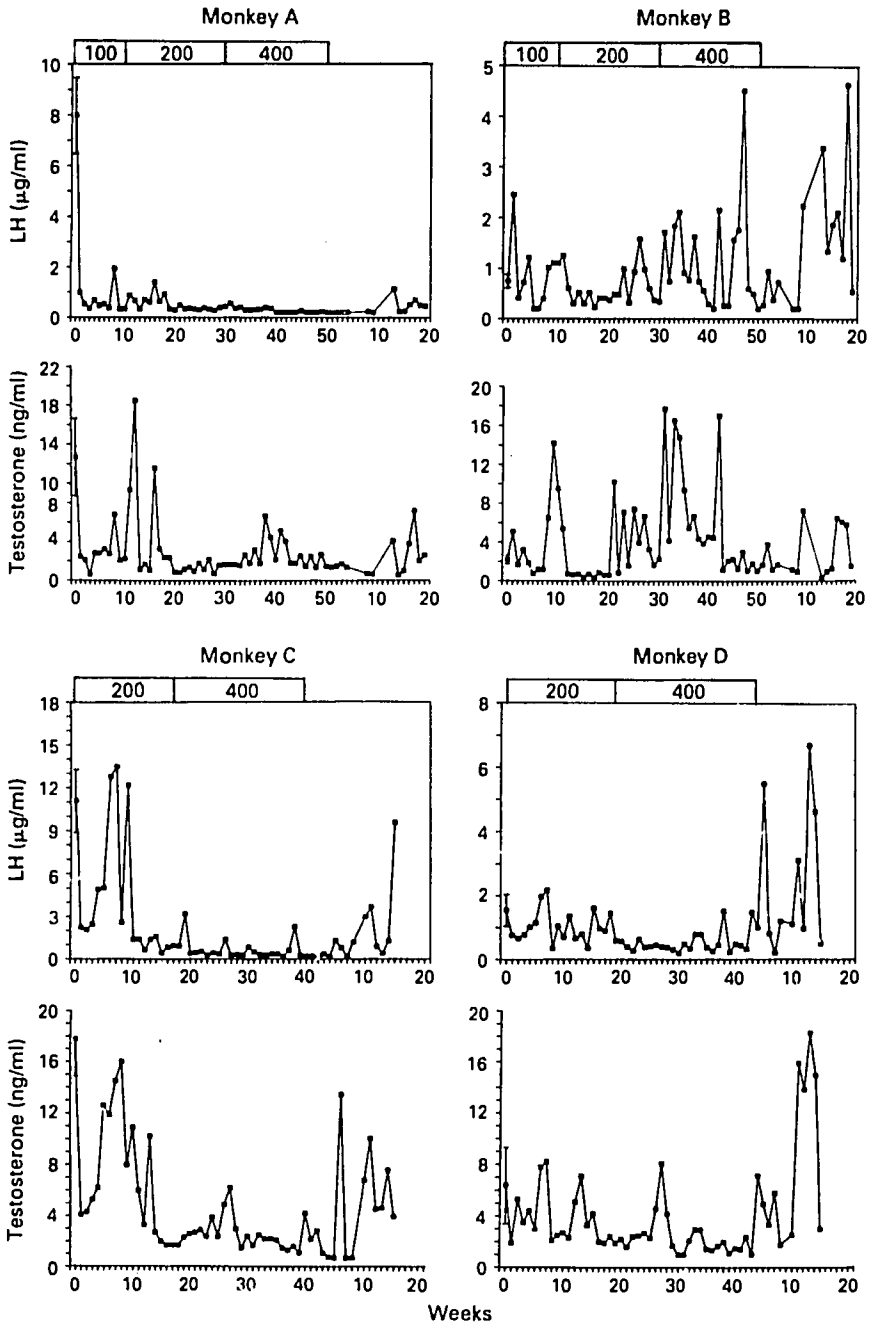

Fig. 1. Influence of continuous GnRH antagonist infusion on serum LH and testosterone concentrations in Monkeys A, B, C and D. The doses of antagonist administered (µg/day) and the length of administration are shown at the top of the figure. The total treatment period was 50 weeks for monkeys A and B and 36 weeks for Monkeys C and D.

recovery. The lack of suppression of LH and testosterone by 100 µg antagonist/day in the Monkeys A and B led to deletion of this treatment in Monkeys C and D and treatment began with 200 µg/day (Fig. 1). Serum LH and testosterone concentrations fell initially, showed some recovery and then declined again to subnormal values.

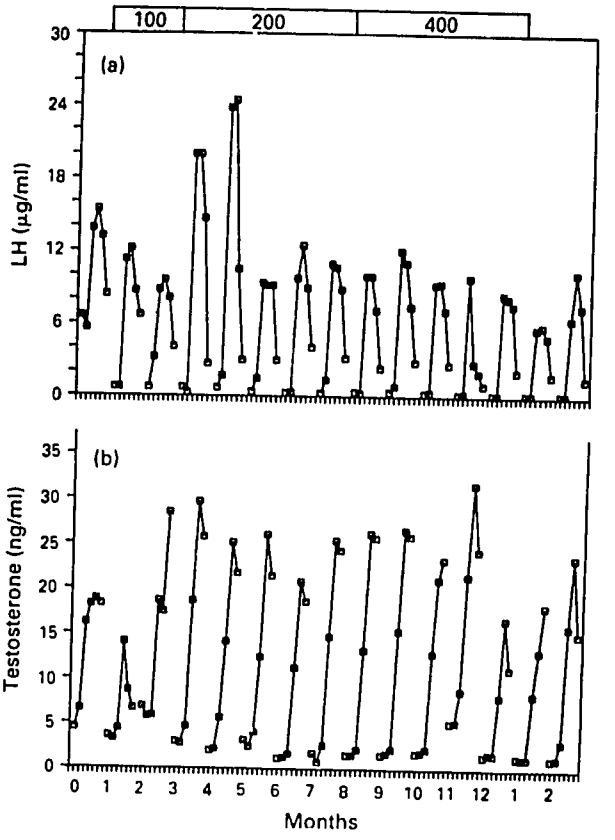

Fig. 2. Typical serum LH (a) and testosterone (b) response to 25 μg GnRH in Monkey A before (0 months), at monthly intervals during 12 months of antagonist administration and at 1 and 2 months of the post-treatment period. Blood samples were taken at −15, 0, +15, +30, +60 and +120 min of GnRH infusion. The doses of antagonist administered (μg/day) and the length of administration are shown at the top of the figure. The total treatment period was 50 weeks.

Figure 2 shows a typical serum LH and testosterone response to a challenge with 25 μg GnRH in Monkey A. The peak serum LH response to GnRH was decreased initially by antagonist treatment, recovered to values that exceeded the response in the pretreatment period, then fell again to values that were about 40% of the pretreatment response when the dose of antagonist was increased. The serum testosterone response to GnRH was elevated above the pretreatment response throughout antagonist administration, suggesting hypersensitivity of the testis to LH. The other 3 animals showed a similar response to GnRH administration.

Mean (±s.e.m.) sperm count decreased ($P < 0{\cdot}05$) over the course of GnRH antagonist infusion (Fig. 3a), from $7{\cdot}1 \pm 2{\cdot}4 \times 10^8$/ml during the pretreatment period to $0{\cdot}29 \times 10^8$/ml during the last month of antagonist infusion. The percentages of motile and live spermatozoa in ejaculates (Fig. 3b) also decreased during the treatment period, but not statistically significantly so.

During the final month of antagonist treatment, spermatozoa from treated monkeys showed a reduced ability ($P < 0{\cdot}005$) to penetrate denuded hamster ova (Fig. 4). Spermatozoa from control animals (age matched; housed under similar conditions to the treated monkeys) showed a mean ± s.e.m. penetration rate of $27{\cdot}7 \pm 4{\cdot}2$% compared with $5{\cdot}5 \pm 2{\cdot}0$% for spermatozoa from antagonist-treated monkeys. When the test was repeated 16 weeks after the end of antagonist treatment, spermatozoa from 3 of 4 treated monkeys showed a normal penetration rate. The sample from the

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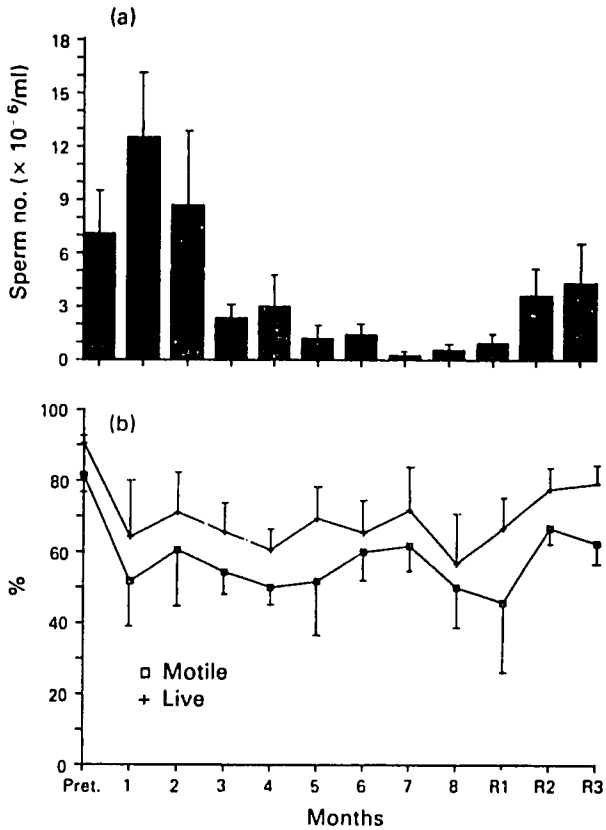

Fig. 3. Effect of continuous GnRH antagonist administration on mean ± s.e.m. sperm count (a) and % motile and % live spermatozoa (b) in the ejaculates of the 4 monkeys. Pret. = pretreatment; R = recovery period.

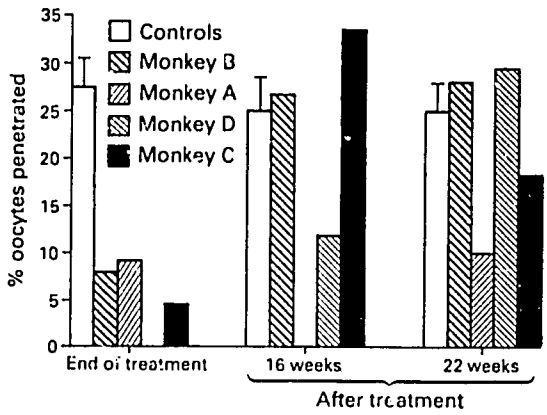

Fig. 4. Effect of GnRH antagonist administration on the mean ± s.e.m. percentage penetration of hamster ova by spermatozoa from untreated control monkeys and from individual animals treated by a GnRH antagonist. The test was run on all monkeys during the last month of treatment, and at 16 and 22 weeks after treatment.

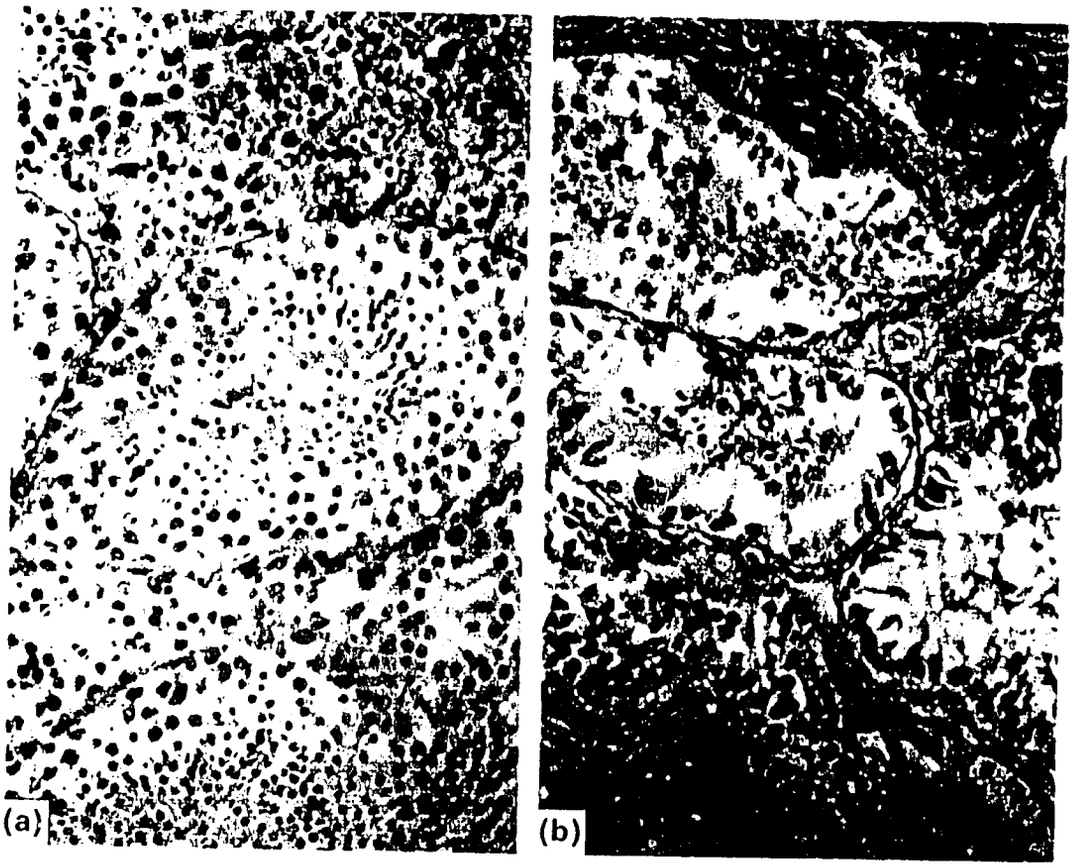

Fig. 5. Effect of GnRH antagonist treatment on testicular histology in Monkey A: (a) before treatment and (b) at the end of treatment. × 200.

4th animal, Monkey A, did not penetrate hamster ova, but when the test was repeated 7 weeks (22 weeks after treatment) later, the penetration rate had increased to 10%. By 26 weeks after the end of treatment, the penetration rate of spermatozoa from Monkey A was 37·5%. This monkey also showed a slower return to normal basal concentrations of serum LH and testosterone after the end of antagonist treatment (see Fig. 1).

In the antagonist-treated monkeys, active spermatogenesis persisted, as indicated by the presence of germ cells representative of all stages of spermatogenesis, including spermatids (Fig. 5), but though present, spermatids were less numerous than in pretreatment biopsies. There was no thickening of the lamina propria, or other evidence of intertubular fibrosis. No areas of calcification were noted.

The testicular volumes of the 4 animals treated with the GnRH antagonist are shown in Fig. 6. Testicular volumes declined more than 45% in the 4 monkeys during antagonist treatment. In Monkey B, in which it was not possible to maintain suppressed concentrations of serum LH and testosterone (see Fig. 1), testicular volume increased between 5 and 8 months of antagonist treatment before declining again.

The mean ± s.e.m. of pretreatment body weights (8·9 ± 0·5 kg) did not differ from body weights (9·4 ± 0·6 kg) at the end of treatment. None of the 4 monkeys lost weight over the course of antagonist administration. We did not observe any effect of antagonist treatment on spontaneous activity nor was there any noticeable peripheral or facial oedema in the monkeys.

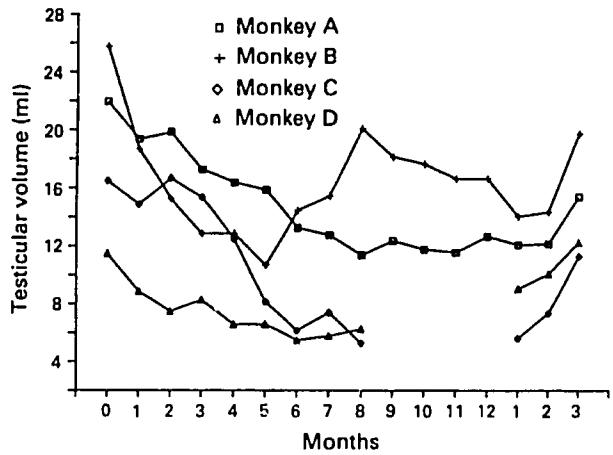

Fig. 6. Effect of GnRH antagonist treatment on testicular volume in the 4 monkeys (Monkeys A and B were treated with antagonist for 12 months, and Monkeys C and D for 8 months). The final 3 measurements were made on each animal at 1, 2, and 3 months after treatment.

Discussion

The inhibitory effect of GnRH antagonists on gonadal function apparently relates to their ability to bind with high affinity to pituitary GnRH receptors, preventing binding by native GnRH (Clayton *et al.*, 1982). There is no evidence that antagonists alter the turnover rate of the GnRH receptor (Bex & Corbin, 1984). In the present study, serum LH and testosterone concentrations showed an immediate fall with the onset of antagonist treatment, but then recovered toward pretreatment values after 8–10 weeks of antagonist administration (100 μg/day). This recovery may result from compensation by the hypothalamus and/or pituitary to the reduced levels of LH, and increased sensitivity of the pituitary to GnRH. Our data support this mechanism. During the periods that LH and testosterone showed recovery during antagonist treatment, the serum LH response to GnRH was supernormal. Furthermore, the serum testosterone response to GnRH was elevated above the pretreatment response throughout antagonist administration, suggesting increased sensitivity of the gonads to LH in the presence of reduced serum concentrations of gonadotrophin.

Two other explanations are possible for the recovery from antagonist treatment. It is possible that antibodies against GnRH were generated, leading to a reduced suppression. This is unlikely from this small decapeptide, although it cannot be excluded because of its highly modified nature relative to native GnRH. A second possibility is that the GnRH antagonist loses its potency during the 4 weeks it is present in the minipump. This explanation can be excluded by the fact that serum LH and testosterone concentrations were not suppressed again when pumps were replaced, but only by increased levels of the GnRH antagonist.

While there was suppression of LH and testosterone in some animals with continuous low doses of the antagonist, the suppression was not complete and serum LH and testosterone values tended to increase after a period of treatment. These results clearly suggest that the mechanism for inhibition by GnRH antagonists is very different from the continuous administration of small amounts of a GnRH agonist. Agonist treatment leads to initial stimulation of LH followed by down regulation of the GnRH receptor and marked suppression of LH concentrations (Heber & Swerdloff, 1981). On the other hand, the antagonists suppress LH by a receptor occupancy mechanism (Clayton *et al.*, 1982).

At the doses of antagonist used in this study, we did not encounter the side effects reported by previous workers (Schmidt *et al.*, 1984; Weinbauer *et al.*, 1984; Akhtar *et al.*, 1985)). Two different GnRH antagonists (*N*-Ac-D-p-Cl-Phe^{1,2}-D-Trp^{3}-D-Arg^{6}-D-Ala^{10}-GnRH and *N*-Ac-D-Nal(2)^{1}-D-

p-Cl-Phe^2-D-Trp^3-D-Arg(Et$_2$)^6-D-Ala^{10}-GnRH) caused a significant loss of weight when administered in daily doses sufficient to induce azoospermia (Weinbauer *et al.*, 1984; Akhtar *et al.*, 1985). The antagonist used in this study was the same analogue which caused oedema in rats and reduced spontaneous activity in monkeys (Schmidt *et al.*, 1984). We did not observe any apparent side effects in our animals, possibly because the doses used were only 5–10% of those previously used and were given at constant rates.

Testicular biopsies performed at the end of antagonist treatment (8–12 months) in the 4 monkeys revealed active spermatogenesis, but decreased numbers of spermatogenic cells in the seminiferous tubules. There was no evidence of tubular calcification or fibrosis. A final needle biopsy performed at 16 weeks of recovery showed that the testicular morphology was fully recovered from antagonist treatment. The effects of the antagonist on testicular histology in monkeys differ markedly from those obtained using a potent GnRH agonist (Mann *et al.*, 1985). The agonist caused a total blockade of spermatogenesis within 20 weeks of treatment with diffuse atrophy of the seminiferous tubules. Tubules contained only a small number of spermatogonia and no evident spermatids. Part of the difference in the effects of the two analogues on spermatogenesis may relate to the degree of suppression of testosterone concentrations. In the antagonist-treated animals, serum testosterone values were suppressed below pretreatment levels but were above the values in agonist-treated monkeys.

The serum testosterone concentration needed to maintain normal spermatogenesis may be greater than that necessary to maintain ejaculatory response. Antagonist administration greatly reduced sperm counts in the present study, but an apparently normal electroejaculatory response was maintained. This suggests that a dose of GnRH antagonist may be found that would induce azoospermia or functional azoospermia (oligospermia with reduced potential fertilizing capacity of remaining spermatozoa) without the undesirable side effects reported by previous investigators (Schmidt *et al.*, 1984; Weinbauer *et al.*, 1984; Akhtar *et al.*, 1985) or loss of ejaculatory response.

Our results show that continuous infusion of 100–400 μg/day of a GnRH antagonist caused oligospermia, although azoospermia was not achieved. These results contrast with those of previous studies in which doses 5–10-fold higher did induce azoospermia (Weinbauer *et al.*, 1984; Akhtar *et al.*, 1985). Additional studies with a higher dose of GnRH antagonist administered by continuous infusion will be necessary to determine whether a dose of GnRH antagonist can be identified which gives azoospermia without undesirable side effects or loss of ejaculatory response. Our study suggests that it may be possible to achieve reliable contraception without attaining azoospermia. Spermatozoa from GnRH antagonist-treated animals penetrated only 6% of denuded hamster ova versus 27% by spermatozoa from untreated animals, suggesting that GnRH antagonist-treated male monkeys have a reduced fertilizing potential.

We thank Wyeth Laboratories, Philadelphia, PA, and the Contraceptive Branch of the National Institutes of Health for the GnRH antagonist used in this study; the National Institute of Arthritis, Diabetes, and Kidney Diseases for providing the rhesus monkey gonadotrophin reference preparation (LER-1909-2); and Ms Charlotte Free, Ms Toni Duffey and Mr Thomas Buckner for technical assistance. The work was supported by NIH grants RR-08006 and RR-00165, Wyeth Laboratories, AID grant DAN-5053-G-SS-5018-00 and Veterans Administration project 1517-001.

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Received 25 March 1987

XN-1 EC-590-B
1506772

J. Reprod. Fert. (1989) 85, 89–95

Printed in Great Britain
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Evaluation of the possible direct effects of gonadotrophin-releasing hormone analogues on the monkey (*Macaca mulatta*) testis

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Summary. In Exp. 1, the effect of treatment with a GnRH agonist on basal concentrations of serum testosterone and peak values of serum testosterone after administration of hCG was determined. One group of adult male monkeys was treated with a low dose (5–10 μg/day) and a second group with a high dose (25 μg/day) of a GnRH agonist for 44 weeks. Basal and peak testosterone concentrations were both significantly reduced by GnRH agonist treatment in all groups compared to untreated control animals, but the % rise in serum testosterone above basal values in response to hCG administration was unchanged by agonist treatment.

In Exp. 2, the GnRH agonist (100 or 400 ng) or a GnRH antagonist (4 μg) was infused into the testicular arteries of adult monkeys. The agonist did not alter testosterone concentrations in the testicular vein or testosterone and LH values in the femoral vein.

In Exp. 3, testicular interstitial cells from monkeys were incubated with three concentrations (10^{-9}, 10^{-7} and 10^{-5} M) of the GnRH agonist or a GnRH antagonist with and without hCG. After 24 h, neither basal nor hCG-stimulated testosterone production was affected by the presence of the GnRH agonist or antagonist.

The results from all 3 experiments clearly suggest that GnRH agonist treatment does not directly alter steroid production by the monkey testis.

Keywords: GnRH; testes; LH; testosterone; rhesus monkey

Introduction

Chronic administration of gonadotrophin-releasing hormone (GnRH) agonists causes azoospermia in monkeys and men (Akhtar *et al.*, 1983a, b; Swerdloff *et al.*, 1985; Mann *et al.*, 1985, 1987). Simultaneous testosterone therapy to maintain libido and potency in men (Swerdloff *et al.*, 1985; Bouchard *et al.*, 1986) and ejaculatory response in monkeys (Mann *et al.*, 1987) does not interfere with agonist-induced azoospermia. Administration of GnRH antagonists also suppresses spermatogenesis in primates although the doses of antagonist required for this effect are much greater than for agonists (Weinbauer *et al.*, 1984; Akhtar *et al.*, 1985). These developments suggest that GnRH analogues may be useful for male contraception.

The mechanism of the antifertility effects of GnRH analogues in primates remains undefined. GnRH agonists reduce the luteinizing hormone (LH) and testosterone response to GnRH (Akhtar *et al.*, 1983b; Mann *et al.*, 1985, 1987), presumably by down-regulating pituitary GnRH receptors, as reported for rats (Heber *et al.*, 1982). GnRH antagonists bind with high affinity to GnRH

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receptors in the pituitary, preventing binding by GnRH (Clayton *et al.*, 1982). In the rat, these analogues also directly inhibit gonadal steroidogenesis (Hsueh & Erickson, 1979; Clayton *et al.*, 1980; Hsueh *et al.*, 1981; Sharpe & Cooper, 1982a, b, 1987; Sharpe, 1982; Spona *et al.*, 1985). However, most evidence does not support a gonadal site of action of these analogues in primates. GnRH agonist treatment does not abolish the testicular response to LH or hCG in primates (Mann *et al.*, 1984; Schaison *et al.*, 1984; Heber *et al.*, 1984; Evans *et al.*, 1984), and GnRH receptors were not demonstrated on the primate gonad (Asch *et al.*, 1981; Clayton & Huhtaniemi, 1982).

Evidence for a direct effect of GnRH or GnRH-like peptides on the primate gonad is supported by findings that the seminiferous tubules of the stump-tailed macaque contain low-affinity GnRH-binding sites and GnRH-like biological acivity, and human seminal plasma has GnRH-like immunoactivity (Sharpe *et al.*, 1981; Swerdloff *et al.*, 1984). In addition, the presence of a GnRH agonist inhibited progesterone secretion from cultured human granulosa cells (Tureck *et al.*, 1982). Progesterone secretion was normal with simultaneous incubation with a GnRH antagonist (Tureck *et al.*, 1982). These results clearly indicate that the question about a direct effect of GnRH agonists on the primate gonad has not been resolved.

In this study, we tested the hypothesis that there is a direct effect of GnRH analogues on the testis of the rhesus monkey. We examined the effect of: (1) chronic GnRH agonist administration (44 weeks) on the testicular response to a low dose of hCG, (2) acute infusion of a GnRH agonist or antagonist into the testicular artery on testosterone release into the testicular vein in adult monkeys and (3) in-vitro incubation of monkey testicular interstitial cells with a GnRH agonist or antagonist on basal and hCG-stimulated testosterone production.

Materials and Methods

The 23 adult male rhesus monkeys (*Macaca mulatta*) used in this study were housed under a 12-h light:12-h dark lighting schedule (lights on: 06:00 h) at 20–23°C.

Experiment 1: effect of chronic GnRH agonist administration on the response to hCG. Ten monkeys were allocated randomly to two groups. Group 1 animals were treated continuously with a GnRH agonist (D-Trp^6-*N*-alpha-Me-Leu^7-desGly^{10}-Pro^9-NHEt-GnRH; Wyeth Laboratories, Philadelphia, PA), 5 µg/day for 24 weeks followed by a dose of 10 µg/day of the same agonist for 20 weeks, using an osmotic minipump as previously described (Mann *et al.*, 1985, 1987). The dose of agonist was doubled from 5 to 10 µg/day after 24 weeks because the 5 µg/day dose was not effective in reducing sperm counts (Mann *et al.*, 1987). Group 2 monkeys received 25 µg agonist/day for 44 weeks. Pumps were replaced at 4-week intervals. All animals were injected i.v. with 50 i.u. hCG during the control (pretreatment) period and at the end of 44 weeks of agonist administration. Blood samples were taken 15 min before and at 0, 30, 60 and 120 min after hCG administration for determination of testosterone by radioimmunoassay.

Experiment 2: influence of acute infusion of a GnRH agonist or antagonist into the testicular artery on serum testosterone concentrations in testicular vein. Eighteen adult male monkeys were used with each animal serving as its own control. Animals were anaesthetized with 30 mg ketamine HCl/kg and prepared for abdominal surgery under sterile conditions. A midline incision was made in the abdominal region and the inguinal canal identified on one side of the animal. The GnRH agonist (100 or 400 ng), a GnRH antagonist (4 µg; Ac-D-pClPhe-D-Trp-Ser-Tyr-D-Arg-Leu-Arg-Pro-D-Ala-NH_2·CH_3COOH; Organon, Oss, The Netherlands) or saline (0·154 M-NaCl) was infused into the testicular artery (proximal to its involvement with the pampiniform plexus) over a 1-min period, and blood samples were taken from the testicular (0·5 ml) and femoral veins (4 ml) before and at 15, 30, 60 and 120 min after administration of the agonist for testosterone and LH determination. Analgesics were administered during the immediate post-surgical period. After a 2–3-month recovery period, animals were subjected to the identical procedure on the other testis.

Experiment 3: effect of GnRH agonist or antagonist treatment on testosterone production from monkey testicular interstitial cells. Testicular interstitial cells were prepared from 2 sexually mature male monkeys (3·5 and 12 years old). The testes were decapsulated and the remaining tissue was minced into small pieces. The tissue was then incubated in Medium 199 (pH 7·4; GIBCO, Grand Island, NY, USA) containing 1·0 mg collagenase/ml and 100 i.u. penicillin–streptomycin/ml in a shaking water bath at 34°C for 45 min under an atmosphere of 5% CO_2:95% O_2. The cell suspension was then filtered through nylon (Nitex, mesh no. HD3-100, Tetko Inc., Elmsforce, NY, USA) and washed 3 times with Medium 199 without collagenase followed each time with centrifugation (400 *g* for 5 min at 4°C). Cells were counted with a haemocytometer and viability was assessed with trypan blue. Cells were rediluted to 10^7 cells per ml in Medium 199 containing 0·1 mM-isobutyl-l-methylxanthine.

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In Exp. 3A, cells (10^6) were incubated with 0, 10^{-9}, 10^{-7} or 10^{-5} M agonist in the absence or presence of hCG (50, 250 or 1000 μi.u.) for 24 h at 34°C under an atmosphere of 5% CO_2:95% O_2 in a shaking water bath. In Exp. 3B, the agonist was replaced with 10^{-9}, 10^{-7} and 10^{-5} M antagonist in the presence or absence of hCG. Viability of cells was reassessed at the end of the incubation period. Cells were removed by centrifugation and testosterone concentration was determined in the incubation medium.

Assays. Serum concentrations of LH were measured using the mouse interstitial cell bioassay (Van Damme *et al.*, 1974). A rhesus monkey pituitary gonadotrophin standard (LER 1909–2) provided by the National Institutes of Diabetes and Diseases of the Kidney was used as the reference preparation. The intra- and interassay coefficients of variation were 11·2% and 14·7% respectively. The usable range of the LH bioassay standard curve was from 0·2 to 10 μg/ml. Serum testosterone concentrations were measured by radioimmunoassay as described in detail previously (Perachio *et al.*, 1977). The intra- and interassay coefficients of variation were 4·8% and 6·7% respectively. The usable portion of the testosterone standard curve was from 2 to 50 nmol/l.

Statistical analysis. Data from Exps 1 and 2 were evaluated by a one-way analysis of variance (agonist treatment) with repeated measures (blood samples over time) followed by the least significant difference test for multiple comparisons. In Exp. 3, data were evaluated by a two-way analysis of variance (agonist or antagonist treatment in the presence or absence of hCG).

Results

Experiment 1: effect of chronic GnRH agonist administration on the response to hCG

Basal serum concentrations of testosterone were reduced in adult male monkeys in Group 1 and 2 compared to basal values during the control (pretreatment) period (Fig. 1). The peak serum testosterone concentration after an i.v. bolus of hCG was <40% of the control peak in Group 1 ($P < 0{\cdot}001$) and <30% in Group 2 ($P < 0{\cdot}001$) (Fig. 1). However, when the lower basal values of testosterone in Groups 1 and 2 after agonist treatment were considered, the overall % increase of testosterone in response to hCG after agonist treatment in Groups 1 and 2 (666% ± 117 and 892% ± 436) did not differ from the control response (652% ± 97).

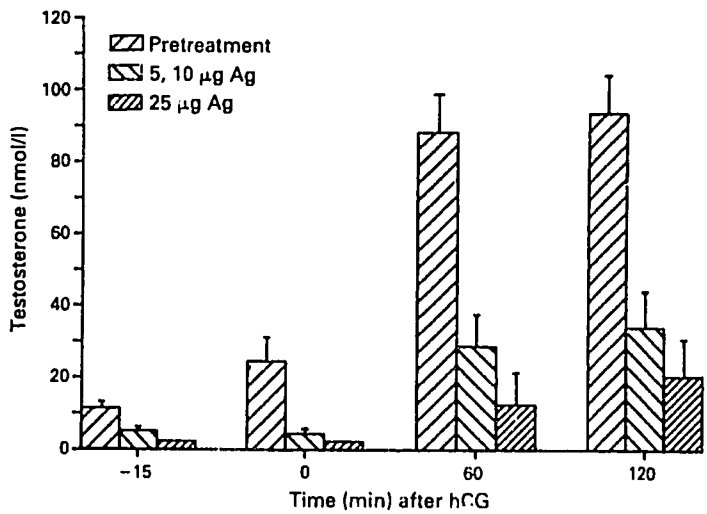

Fig. 1. Serum testosterone concentrations before and after hCG administration in adult male monkeys. Ag = agonist. Group 1 (N = 5): GnRH agonist, 5 μg/day for 24 weeks and then 10 μg/day for 20 weeks. Group 2 (N = 5): GnRH agonist, 25 μg/day for 44 weeks. Animals in Groups 1 and 2 had lower basal values ($P < 0{\cdot}05$) and reduced peak values of serum testosterone after hCG ($P < 0{\cdot}001$) than during the pretreatment period.

Experiment 2. influence of acute infusion of GnRH agonist or antagonist into the testicular artery on testosterone concentrations in the testicular and femoral veins

Infusion of 100 or 400 ng GnRH agonist into the testicular artery failed to change testosterone concentrations in the testicular vein or systemic circulation over the next 2 h (Table 1). The infusion of 4 μg GnRH antagonist also failed to influence testicular secretion of testosterone into the testicular vein (Table 1).

Table 1. Effect of infusion (over 1 min) of 100 or 400 ng GnRH agonist or 4 μg antagonist into the testicular artery on testosterone concentrations in the testicular vein and on LH and testosterone concentrations (±s.e.m.) in the femoral vein in adult monkeys

	Time (min) after agonist or saline infusion					
	−15	0	15	30	60	120
	Testicular vein testosterone (nmol/l)					
Control	198 ±71	189 ±52	156 ±42	170 ±38	125 ±34	90 ±20
Agonist						
100 ng	183 ±87	237 ±116	157 ±74	141 ±64	253 ±180	130 ±44
400 ng	135 ±41	127 ±40	234 ±71	271 ±70	304 ±99	122 ±29
Antagonist						
4 μg	193 ±124	202 ±103	155 ±53	194 ±44	186 ±40	135 ±69
	Femoral vein testosterone (nmol/l)					
Control	15 ±5	21 ±7	17 ±6	19 ±6	16 ±5	12 ±4
Agonist						
100 ng	24 ±9	18 ±5	15 ±3	14 ±3	15 ±5	11 ±2
400 ng	14 ±4	15 ±4	18 ±2	17 ±3	17 ±3	16 ±3
Antagonist						
4 μg	11 ±2	12 ±2	13 ±2	14 ±2	14 ±2	11 ±1
	Femoral vein LH (μg/ml)					
Control	3·2 ±0·5	3·3 ±0·5	2·7 ±0·4	2·7 ±0·5	1·9 ±0·3	1·2 ±0·2
Agonist						
100 ng	3·5 ±1·1	3·6 ±1·2	3·9 ±1·2	4·1 ±1·4	3·0 ±0·9	1·8 ±0·5
400 ng	4·4 ±0·8	4·4 ±0·6	6·8 ±1·6	6·2 ±1·9	6·3 ±1·7	2·9 ±0·7
Antagonist						
4 μg	3·0 ±0·8	3·3 ±0·9	3·5 ±1·3	2·5 ±0·8	2·1 ±0·7	1·6 ±0·5

Values are mean ± s.e.m.; N = 6 for each GnRH analogue treated group and each animal served as its own control.

Experiment 3: effect of a GnRH agonist or antagonist on testosterone production from monkey testicular interstitial cells in vitro

As shown in Table 2, incubation of testicular interstitial cells with 10^{-9}, 10^{-7} or 10^{-5} M-GnRH agonist or antagonist failed to alter the amount of testosterone in the incubation medium after 24 h;

testosterone production ranged from 4·4 and 4·7 pmol in Exp. 3A and 1·0 to 1·2 pmol in Exp. 3B. Untreated interstitial cells showed a dose-related increase ($P < 0{\cdot}001$) in testosterone production when incubated with increasing doses of hCG (50–1000 μi.u.) in Exps 3A and 3B. While basal testosterone production was lower in Exp. 3B than in Exp. 3A, the overall response of cells (based on the % increase over basal values) to hCG did not differ between the two experiments (743% and 745%). The response of these cells to hCG was not affected by the presence of the GnRH agonist or antagonist in the incubation medium.

Table 2. Effect of a GnRH agonist and antagonist on testosterone production (pmol/10^6 cells/24 h) by isolated testicular interstitial cells from male monkeys

		hCG dose (μi.u.)			
Exp.	Treatment	0	50	250	1000
3A	Control	4·7 ±0·5	7·4 ±1·0	18·9 ±1·0	30·0 ±1·8
	Agonist				
	10^{-9} M	4·6 ±0·4	7·0 ±0·5	19·4 ±0·7	34·2 ±2·3
	10^{-7} M	4·5 ±0·4	7·5 ±0·5	24·5 ±2·0	32·9 1·4
	10^{-5} M	4·4 ±0·4	7·5 ±0·5	21·0 ±2·8	33·6 ±2·0
3B	Control	1·1 ±0·1	2·0 ±0·1	6·0 ±0·4	8·2 ±0·5
	Antagonist				
	10^{-9} M	1·0 ±0·1	1·7 ±0·2	3·9 ±0·2	9·6 ±0·4
	10^{-7} M	1·2 ±0·1	1·6 ±0·1	4·5 ±0·3	7·4 ±0·4
	10^{-5} M	1·0 ±0·1	1·4 ±0·1	4·7 ±0·2	8·7 ±0·7

Values are mean ± s.e.m. of at least 5 incubates.

Discussion

It has been clearly established that GnRH analogues have direct effects on testicular function in the rat. In hypophysectomized rats, GnRH agonists inhibited steroidogenesis, reduced the responsiveness to LH, and blocked FSH induction of LH receptors on Leydig cells (Bambino *et al.*, 1980; Hsueh *et al.*, 1981). In-vitro incubation of testicular interstitial or purified Leydig cells with GnRH agonist had a biphasic effect on testosterone production. Acutely, GnRH agonists increased testosterone production. However, the effect on testicular steroidogenesis was inhibitory in long-term incubations (24–27 h) (Hsueh *et al.*, 1981; Sharpe & Cooper, 1982a). Binding sites for GnRH were reported on rat Leydig cells (Hsueh & Erickson, 1979; Clayton *et al.*, 1980), and GnRH-like factors have been demonstrated in the rat testis (Sharpe *et al.*, 1981; Swerdloff *et al.*, 1984). These data support the hypothesis that there is a GnRH-like gonadal peptide involved in modulating testicular steroidogenesis in the rat testis.

The results reported here do not demonstrate a direct effect of a GnRH agonist or antagonist on testicular steroidogenesis in the primate testis. Chronic GnRH agonist treatment for 44 weeks inhibited spermatogenesis and reduced basal concentrations of testosterone (Mann *et al.*, 1987),

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but failed to alter the testicular testosterone response (based on the % increase of serum testosterone after hCG to the basal testosterone value) of these animals to a low dose of hCG. However, absolute peak concentrations of testosterone after hCG administration were lower in agonist-treated animals than they were during the control period.

Infusion of 100 or 400 ng GnRH agonist or 4 μg antagonist directly into the testicular artery did not significantly alter testosterone concentrations in the testicular vein over the next 2 h. However, serum LH concentrations in animals treated with 400 ng agonist were slightly elevated 15 and 30 min after agonist administration, suggesting that this dose of agonist approaches levels that would stimulate pituitary LH secretion, and therefore, testicular testosterone secretion indirectly.

Finally, in-vitro incubation of testicular interstitial cells from sexually mature male monkeys with the GnRH agonist or antagonist (10^{-9} to 10^{-5} M) for 24 h did not affect either testosterone production or the testosterone response to hCG stimulation. These results do not rule out the possibility that shorter or longer term incubations of the cells with the analogues would have influenced steroidogenesis. However, GnRH agonist treatment of male monkeys for 44 weeks did not alter the testicular testosterone response to a low dose of hCG *in vivo*. Therefore, the in-vivo and in-vitro evidence from the present study does not support the hypothesis that GnRH analogues directly alter testicular steroidogenesis.

The results reported here provide the first direct evidence *in vivo* and *in vitro* that GnRH analogues do not directly alter testicular steroidogenesis in primates. These data are in agreement with a recent report that showed chronic GnRH agonist treatment for 1 year of men with prostatic cancer resulted in an inhibition of the production of Δ^4 and Δ^5 steroids in the testis. This inhibition was overcome by treatment with exogenous hCG (Rajfer *et al.*, 1987). Thus, most evidence does not support the hypothesis that the testis is a site for the inhibitory effects of GnRH agonists on gonadal function. Nevertheless, there is a need to be cautious. Data continue to accumulate suggesting the presence of a GnRH-like protein in the primate gonad (human ovary) (Aten *et al.*, 1987). Moreover, while monkey testicular interstitial cells incubated for 24 h with a GnRH agonist or antagonist failed to show any change in testosterone production in the present study, it is possible that more chronic treatment with the analogues would have altered either basal testosterone secretion or the response of these cells to hCG.

We thank Wyeth Laboratories and Organon for the GnRH agonist and antagonist used in this study; the NIADDK for providing the rhesus monkey pituitary reference preparation (LER-1909-2); Dr Gordon Niswender for the testosterone antiserum; and Ms. Toni Duffey, Janice Tittle, Robin King and Mr Thomas Buckner for technical assistance. Supported by AID grant DAN-5053-G-SS-5018-00, NIH grants RR-08006 and RR-00165 and Veterans Administration project 1517-001.

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Received 6 April 1988

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0021-972X/87/6506-1215$02.00/0
Journal of Clinical Endocrinology and Metabolism
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XN-ARC-590-C
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Vol. 65, No. 6
Printed in U.S.A.

Influence of Simultaneous Gonadotropin-Releasing Hormone Agonist and Testosterone Treatment on Spermatogenesis and Potential Fertilizing Capacity in Male Monkeys*

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ABSTRACT. We examined the effect of continuous sc infusion of a GnRH agonist (Ag) and testosterone (T) supplementation on spermatogenesis and the potential fertilizing capacity of sperm in 15 rhesus monkeys. The monkeys were divided into 3 groups of 5 animals each. Groups 1 and 2 received 25 μg/day Ag for 44 weeks. Group 2 also received T replacement therapy [sufficient to maintain serum T values within the normal range; 4–5 ng/mL (13.9–17.3 nmol/L)]. Group 3 received a low dose of the Ag (5 μg/day for 24 weeks and then 10 μg/day for 20 weeks) to prolong the oligospermic phase before the onset of azoospermia. In groups 1 and 3, there was an initial transient (1 week) rise in serum LH and T levels which then fell below pretreatment values, where they remained throughout the treatment period. The serum LH and T levels were lower in the group treated with 25 μg/day Ag. Similar changes in serum LH levels occurred in group 2, but T supplementation maintained serum T in the physiological range. The decline in serum LH levels was associated with reduced sensitivity to GnRH, since the responses of serum LH and T to GnRH were either abolished or greatly reduced in the 2 groups treated with 25 μg/day Ag and were less than 50% of the pre-Ag responses in monkeys treated with 5–10 μg/day Ag.

Four of five monkeys treated with 25 μg/day Ag alone became azoospermic within 21 weeks. All five animals receiving Ag and T supplementation became azoospermic (mean time to onset, 12.6 weeks). Four of five monkeys treated with 5–10 μg/day Ag also had azoospermic ejaculates during the late treatment and early recovery period. Sperm counts recovered to the pretreatment levels in most monkeys by 10 weeks of the recovery period. The quality of semen samples taken from oligospermic monkeys was greatly reduced. The percentage of motile and percentage of live sperm per ejaculate, the net negative surface charge on sperm, and the scores of sperm in the hamster oocyte penetration test were subnormal. T supplementation did not improve these measures of semen quality. Testicular biopsies taken at the end of the Ag administration period from monkeys given 25 μg/day Ag showed diffuse atrophy of the seminiferous tubules, which contained primarily Sertoli cells and a few spermatogonia and spermatocytes, but no spermatids. The tubular atrophy and the suppression of spermatogenesis did not appear to be influenced by T replacement. These results do not support the conclusion of other investigators that T supplementation in the Ag-treated monkey prevents the induction of azoospermia. The data suggest that GnRH analog administration, combined with androgen therapy to maintain libido and potency, may be an acceptable method of male contraception. (*J Clin Endocrinol Metab* **65:** 1215, 1987)

WE AND others have found that continuous infusion of GnRH agonists (Ag) induced azoospermia in monkeys (1, 2), and that spermatogenesis could not be reinitiated by testosterone (T) administration to maintain ejaculatory behavior (1). However, T treatment initiated at the beginning of Ag administration attenuated the effect of the agonist on spermatogenesis (3). Combined treatment induced oligospermia in 8–10 weeks, but azoospermia was not achieved by 22 weeks. Moreover, the decline in testicular volume associated with Ag administration was postponed by T therapy (4). In the context of male contraception, these results suggested that T administration could begin only after azoospermia was achieved, and they thus raised important questions about the feasibility of using GnRH analogs for male contraception.

In men Ag administration not only greatly reduces serum T levels but also causes symptoms of hypogonadism (hot flushes, loss of libido and potency) (5). Androgen therapy is necessary to maintain libido and potency.

Received April 13, 1987.
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* This work was supported by AID Grant DAN-5053-G-SS-5018-00, NIH Grants RR-08006 and RR-00165, and V.A. project 1517-001.

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Combined Ag and androgen treatment of six men for 20 weeks caused oligospermia, but not azoospermia (6). On the other hand, T administration potentiated the inhibitory effect of Ag in rats (7, 8). These conflicting data and the need for androgen replacement during Ag-induced infertility made it important to reexamine whether combined Ag and T administration would induce azoospermia in the primate. In this study, therefore, we tested the effect of administration of Ag with and without T replacement therapy on reproductive function in monkeys. A second objective of this study was to determine the potential fertilizing capacity of sperm from Ag-treated oligospermic monkeys, since we recently found that sperm that appeared normal in morphology and motility from GnRH antagonist-treated animals may have reduced fertilizing potential (2).

Materials and Methods

Fifteen adult male monkeys (*Macaca mulatta*), ranging in weight from 6.7–10.9 kg and housed singly in a controlled environment (temperature, 20–23 C; lighting schedule, 12 h of light, 12 h of dark), were used in this study.

Treatment groups

The 15 monkeys were randomly assigned to one of three treatment groups of five animals each. Animals in group 1 were treated with 25 μg/day Ag (D-Trp^6-*N*-α-Me-Leu^7-des Gly^{10}-Pro^9-NH Et GnRH; Wy-40972, Wyeth Laboratories, Philadelphia, PA) for 44 weeks using Alzet osmotic minipumps (2ml-4, Alza Corp., Palo Alto, CA). Those in group 2 were treated simultaneously with 25 GnRH Ag, and T was administered using a sc implanted 5-cm long Silastic elastomere (od, 0.465 cm; id, 0.335 cm). One elastomere of T was implanted in each animal. Group 3 received 5 μg/day Ag for 24 weeks and then 10 μg/day Ag for an additional 20 weeks using minipumps. These low Ag dosages were chosen to produce prolonged oligospermia before the onset of azoospermia, so that the potential fertilizing capacity of recovered sperm could be more fully assessed.

Surgical implantation of minipumps and T elastomers

Animals were anesthetized with 10 mg/kg BW ketamine HCl, and the minipumps and T capsules were implanted sc in the interscapular region under sterile conditions. Minipumps containing the appropriate Ag concentration dissolved in sterile water were replaced every 28 days, and T implants were replaced at 16-week intervals. The residual Ag volume in each minipump was measured when it was removed. The mean residual volume was 0.24 ± 0.01 (SEM) mL. This volume did not differ significantly between treatment groups or individual animals.

Blood and semen sampling schedule

Blood samples were taken at weekly intervals during a 5-week control period, on treatment days 2 and 7, and then weekly throughout the 44-week Ag administration and the 20-week recovery periods.

Semen samples were collected by penile electrostimulation. Three to five semen samples were collected from each animal during the control period and at 2-week intervals during the Ag administration and recovery periods for determination of sperm counts, percentage of motile sperm, and percentage of live sperm.

Net surface charge on sperm

Sperm samples were washed, fixed in 2% glutaraldehyde, and exposed to colloidal iron on carbon plates, as previously described (9). The specimens were examined at 25 kV at a 35° takeoff angle in an ISI DS 130 scanning electron microscope (International Scientific Inst., Miltitas, CA). The energy dispersive x-ray spectra of the sperm head were obtained using a Tracor Northern 5500 analyser. The energy spectrum of the x-rays emitted, characteristic of an element, was used to identify the elements present. The peaks derived from Na (1.041 keV), P (2.015 keV), S (2.308 keV), Cl (2.622 keV), Ca (3.691 keV), and Fe (6.403 keV) were quantitated, and the background was subtracted. Thus, the integrals were expressed as a percentage of Fe counts relative to those of the other elements (Fe/Na, P, K, Cl, S, Ca × 100). Duplicate samples were analyzed, and 100 sperm were measured in each sample.

Hamster ova penetration assay (SPA)

The SPA was performed basically as described previously (10) on sperm samples taken during the control period; at 3–10, 11–15, and 16–44 weeks of Ag administration; and at 20 and 25 weeks during the recovery period. The original method was modified by the use of Dulbecco's medium during preparation of the oocytes and by extending the incubation time before reading the assay results. Briefly, oocytes were recovered from superovulated hamsters (25 IU PMSG, ip, followed by 50 IU hCG, ip, 3 days later), the cumulus cells were removed by incubation in 1% hyaluronidase for 15 min, and the zonae were dissolved by transferring the oocytes to a 0.1% trypsin solution in Dulbecco's medium for 10 min. After the zonae were dissolved, the denuded oocytes were transferred to Ham's F-10 medium containing 10% human cord serum. After washing, 10–30 oocytes were incubated in 0.5 mL culture medium in an 8-well culture slide; each well had a volume of 0.6 mL. The oocytes were incubated with 25,000 motile sperm/oocyte in 5% CO_2 in air at 37 C for 17 h. Semen samples were washed twice in Ham's F-10 medium, concentrated by centrifugation, and subjected to the "swim up" technique before incubation with oocytes. The assay was scored according to the percentage of oocytes penetrated per well.

Pituitary and testicular responses to GnRH

During the control period and at 4-week intervals throughout Ag administration and recovery periods, animals were anesthetized with 10 mg/kg BW ketamine HCl and injected iv with 25 μg GnRH. Blood samples were taken at 15 and 0 before and 15, 30, 60, and 120 min after GnRH administration for LH and T determinations.

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Testicular volumes and biopsies

Testicular volume was determined at monthly intervals, as previously described (2). Testicular needle biopsies were performed on one testis from each animal during the control period and on the alternate testis at the termination of Ag administration for routine histological examination, as previously described (2).

Assays for LH and T

Serum LH concentrations were measured in duplicate using the mouse interstitial T-cell bioassay (11). The NIDDK rhesus monkey pituitary gonadotropin standard (LER-1909-2) was used as the reference preparation. The intra- and interassay coefficients of variation for the LH assay were 7.3% and 19.3% for a serum pool containing 2.0 μg/mL LH. The coefficients of variation did not differ significantly over the usable portion of the LH standard curve (0.2–10 μg/mL). The sensitivity of the LH assay was 0.2 μg/mL. Serum T concentrations were measured in duplicate by RIA, as previously described (12). The intra- and interassay coefficients of variation for the T assay were 4.9% and 6.8%, respectively, for a serum pool containing 5 ng/mL T. The sensitivity of the T assay was 0.5 ng/mL.

Statistics

The data were evaluated using either analysis of variance or χ^2 test (SPA, frequency of electroejaculation).

Results

Animals in group 1 were given 25 μg/day Ag for 44 weeks. The animals in group 2 were treated simultaneously with 25 μg/day Ag and T for 44 weeks, and the group 3 animals were given 5 μg/day Ag for 24 weeks, then 10 μg/day Ag for an additional 20 weeks.

Serum LH and T concentrations

Serum LH levels rose initially and then fell precipitously to near-minimal levels of detection (0.2 μg/mL) in four of the five monkeys in group 1 (Fig. 1A, *top*). In these four monkeys, the serum LH levels then remained below the control levels through the remainder of the Ag administration period. The fifth animal (no. 4960; data not included in Fig. 1) was less sensitive to the Ag. The pattern of change in serum LH concentrations was similar, but the LH values remained near-control values during the last 40 weeks of Ag administration. Serum T values in the four responsive monkeys rose from control values between 4 and 8 ng/mL (13.9–27.7 nmol/L) to 39.7 ± 5.6 (±SEM) ng/mL (138 ± 19 nmol/L) on day 2, and then declined to near-castrate values (<0.70 ng/mL; 2.4 nmol/L; Fig. 1A, *bottom*). The fifth animal (no. 4960) had normal serum T levels after the initial increase. In the group 2 monkeys, the changes in serum LH were similar to those in the four responsive monkeys in group 1. The serum T pattern was similar, except serum T did not fall to castrate values, but was maintained between 4–5 ng/mL (13.9–17.3 nmol/L) by the sc T implants (Fig. 1B). The monkeys in group 3 had an initial rise in serum LH and T levels similar to that in group 1 (Fig. 1C), but there was less suppression of serum LH and T levels thereafter.

Serum LH and T concentrations increased soon after the termination of Ag administration and were similar to pretreatment values within 10–12 weeks (Fig. 1). Serum LH and T levels were supranormal for 10 weeks in groups 1 and 2 (Fig. 1, A and C). The group 3 animals had supranormal serum LH but normal serum T levels during this period (Fig. 1C).

Pituitary and testicular responses to GnRH

The serum LH and T responses to 25 μg GnRH were abolished in four of the five monkeys in group 1 and the five animals in group 2 (Fig. 2). The remaining animal (no. 4960) in group 1 had significant, but reduced, serum LH and T responses to GnRH administration throughout the Ag administration period (data not shown). In the animals in group 3, the peak serum LH and T responses to GnRH were reduced to less than 30% of the control responses by 36 weeks (Fig. 2). The serum LH and T responses to GnRH had returned to the control levels in all groups within 8 weeks after the discontinuation of Ag.

Testicular volumes

There was a significant effect of time ($P < 0.005$) and a significant interaction ($P < 0.05$) of the two variables (time × treatment) on testicular volume. An initial small increase in testicular volumes was found after 2 weeks of Ag administration, followed by a rapid decline to a plateau after 10 weeks in all three groups (Fig. 3). The decline (44%) was less marked in group 3 animals than in those in group 1 (71%) or group 2 (73%). Thus, T administration did not alter the Ag-induced decline in testicular volume. The data in Fig. 3 do not include the monkey (no. 4960) that was less sensitive to Ag. This monkey had only a minimal decrease in testicular volume during Ag administration.

Semen analysis

During the pretreatment period the success rate of electroejaculation ranged between 94–95% for the three groups of monkeys. This rate declined in all three groups during the treatment period (Table 1). However, only one of the animals treated simultaneously with Ag and T (group 2) had a reduced frequency of ejaculation. This occurred after 27 weeks of treatment.

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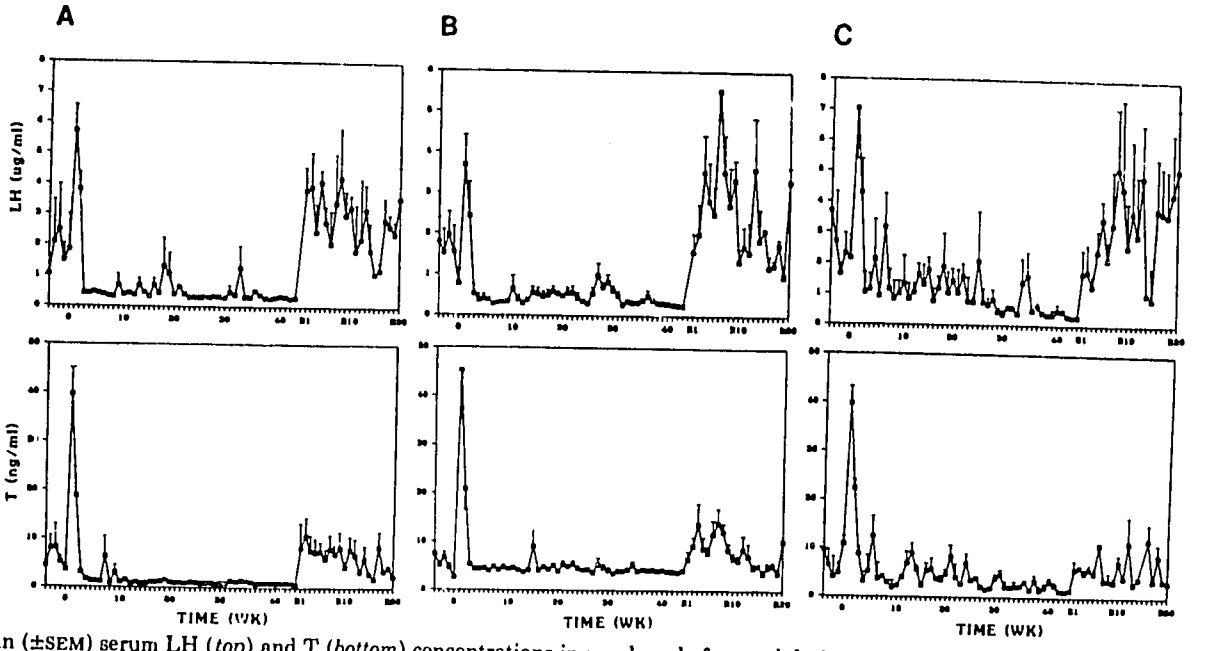

FIG. 1. Mean (±SEM) serum LH (*top*) and T (*bottom*) concentrations in monkeys before and during 44 weeks of Ag administration and during a 20-week recovery (R) period. A, Group 1 animals given 25 μg/day Ag; B, group 2 animals given 25 μg/day Ag and T; C, group 3 animals given 5 μg/day Ag for 24 weeks and then 10 μg/day Ag for 20 weeks. For LH, ng/mL = μg/L. To convert T concentrations from ng/mL to nmol/L, multiply by 3.467.

Figure 4 illustrates the effect of Ag administration on mean sperm counts in the three groups. Four of five animals in group 1 became azoospermic after 11–21 weeks of Ag administration (Fig. 4, *top*). It was difficult to determine exactly when azoospermia was achieved in this group because two of the monkeys did not have a consistent ejaculatory response before the onset of azoospermia. The fifth animal (no. 4960) in this group never had azoospermia, although one azoospermic sample was collected during the recovery period. All five monkeys in group 2 became azoospermic after 16 weeks [mean, 12.6 ± 1.2 (±SEM) weeks], and four of the monkeys consistently had azoospermic ejaculates for the remainder of the treatment period (Fig. 4, *middle*). The fifth animal had no ejaculatory response between weeks 27 of treatment and week 1 of the recovery period. His ejaculates were azoospermic for 12 weeks before and 4 weeks after this loss of ejaculatory response. After azoospermia was achieved in the monkeys in group 2, there were a few occasions (one or two per monkey) when ejaculates had a small number of sperm (<6% of the control level). These ejaculates were of reduced volume (<10% of normal) and contained a subnormal number of live sperm (<30%).

In eight of the nine monkeys in groups 1 and 2 who achieved azoospermia, azoospermia persisted for 7 weeks after the termination of Ag (Fig. 4). After that, sperm counts in all but one animal returned quickly to control levels. The remaining monkey was unresponsive to penile electrostimulation between weeks 8 and 16 of the recovery period. An ejaculate collected during recovery week 17 using the rectal probe electroejaculatory technique contained a normal sperm number (2.4×10^8 million/mL) and normal percentages of motile and live sperm.

Figure 4 (*bottom*) shows the mean sperm counts in the five monkeys in group 3. Mean sperm counts were lower than control levels during the latter part of Ag administration and early in the recovery period, and ejaculates with no sperm were recovered from four of five monkeys in this group during these times.

Group 1 and group 2 animals had mean percentages of motile sperm of 87.1 ± 1.9% (±SEM) and 81.9 ± 1.9%, respectively, and mean percentages of live sperm of 90.4 ± 1.6% (±SEM) and 87.6 ± 1.5%, respectively, during the control period. In the two ejaculates immediately preceding the onset of azoospermia, the percentages of motile and live sperm in the two groups were 38.7 ± 9.4% and 42.5 ± 10.5% ($P < 0.001$) and 53.0 ± 8.9% and 47.2 ± 9.3% ($P < 0.001$), respectively. Monkeys in group 3 also had a reduction in the percentages of motile ($P < 0.01$) and live sperm ($P < 0.01$) in ejaculates during the last 12 weeks of the treatment period (mean percentage of motile sperm: control, 81.9 ± 2.3%; Ag administration, 53.5 ± 9.5%; mean percentage of live sperm: control, 88.5 ± 1.5%; Ag administration, 54.0 ± 9.1%).

The effect of Ag administration on the percentage of hamster oocytes penetrated by sperm from individual monkeys in the 3 treatment groups is shown in Fig. 5. Semen samples from groups 1 and 2 during treatment weeks 11–44 showed a reduced ability to penetrate

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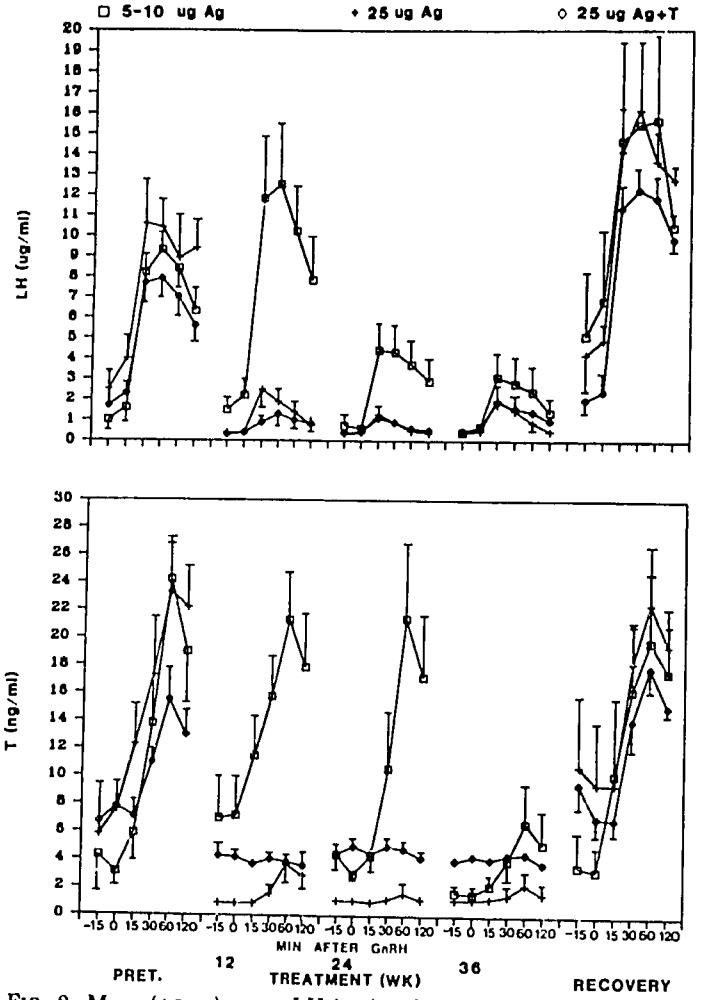

FIG. 2. Mean (±SEM) serum LH (*top*) and T (*bottom*) responses of 14 monkeys to an iv bolus dose of 25 μg GnRH during the control period (PRET), at 12-week intervals during administration of 25 μg/day Ag (group 1), 25 μg/day Ag and T (group 2), or 5–10 μg/day Ag (group 3), and at 8 weeks of the recovery period. For LH, ng/mL = μg/L. To convert T concentrations from ng/mL to nmol/L, multiply by 3.467.

hamster oocytes compared to control samples (by χ^2, $P < 0.05$ for group 1 and $P < 0.005$ for group 2). Overall, there was no significant change in the SPA for group 3. The SPA was performed on semen samples from all monkeys again at 20 weeks of the recovery period. By that time, sperm samples from 8 of 10 monkeys in groups 1 and 2 had a normal penetration score. The 2 monkeys with a subnormal SPA score at 20 weeks of recovery were retested 5 weeks later and had a normal SPA score.

There was an unimodal distribution of net negative surface charge on rhesus monkey sperm (Fig. 6). The observed distribution of the number of sperm showing a given percentage of iron did not differ from the calculated normal distribution when tested by χ^2. The predominant charge of the sperm samples taken during the control period, expressed as the percentage of iron bound to the surface relative to the other elements, was 48.8 ± 1.7%. Ag administration caused a reduction in net negative

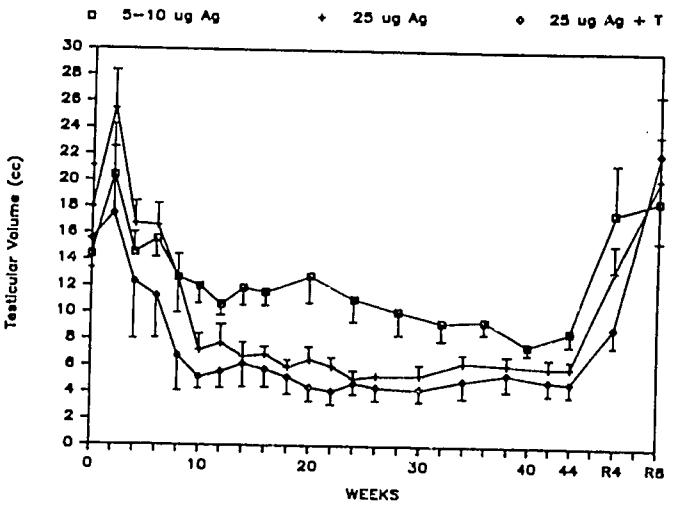

FIG. 3. Mean (±SEM) testicular volume in three groups of monkeys during the control period (0), at 2- or 4-week intervals during the administration of 25 μg/day Ag (group 1), 25 μg/day Ag and T (group 2), or 5–10 μg/day Ag (group 3), and at 4 and 8 weeks of the recovery (R) period.

TABLE 1. Effect of Ag and T administration on the percent success of penile electroejaculation

	Pretreatment	Treatment (weeks)				Recovery
		1–12	13–24	25–36	37–44	
Group 1, 25 μg/day Ag (n = 5)	94	96	70[a]	47[b]	50[c]	91
Group 2, 25 μg/day Ag + T (n = 5)	95	93	100	83	63[d]	87
Group 3, 5–10 μg/day Ag (n = 5)	95	90	80	71	47[e]	87

[a] Significantly different from the pretreatment value, $P < 0.025$.
[b] Significantly different from the pretreatment value, $P < 0.005$.
[c] Significantly different from the pretreatment value, $P < 0.005$.
[d] Significantly different from the pretreatment value, $P < 0.05$.
[e] Significantly different from the pretreatment value, $P < 0.005$.

charge on sperm in all three treatment groups. In animals in group 3, the percentage of iron on the surface declined from a control level of 48.8 ± 4.4% to 27.4 ± 2.1% ($P < 0.001$), compared to a decrease from 45.8 ± 2.2% and 51.7 ± 2.6% to 33.9 ± 4.5% ($P = 0.045$) and 33.0 ± 3.2% ($P = 0.001$) in groups 1 and 2, respectively. T maintenance therapy did not alter the effect of Ag on the net surface charge on sperm.

Testicular histology

Animals in groups 1 and 2 had severe hypospermatogenesis after 44 weeks of Ag administration comparable to that previously reported after short term (20 week) treatment with the same GnRH analog (2). While the degree of inhibition of spermatogenesis varied from mon-

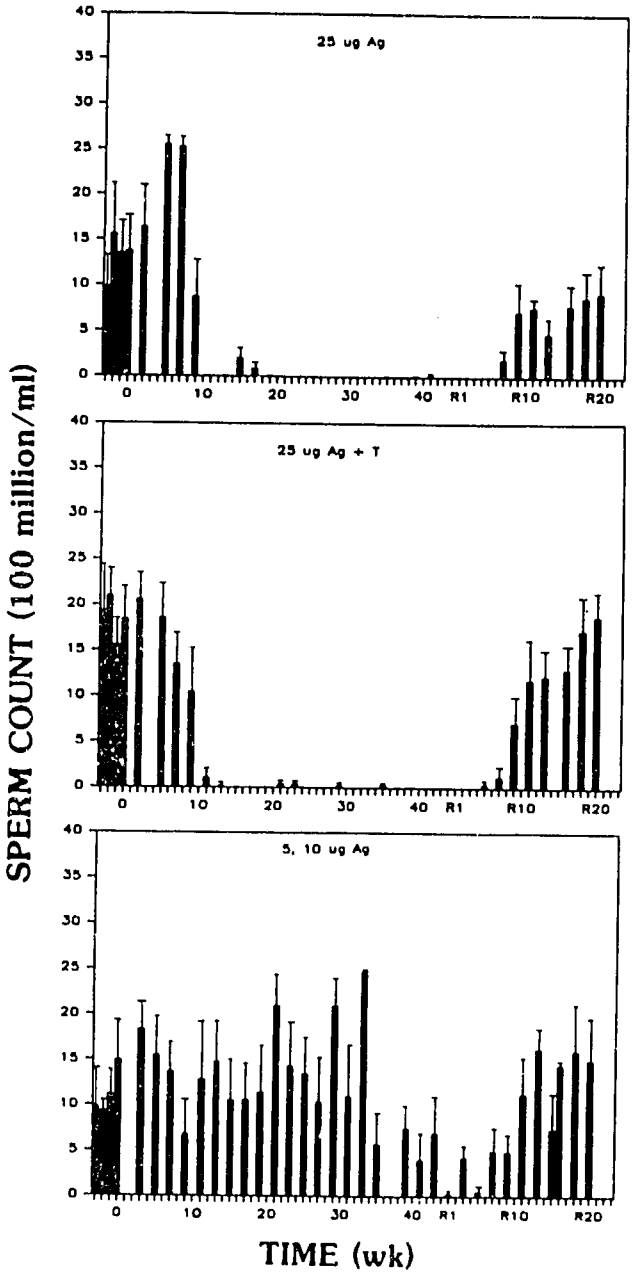

FIG. 4. Mean (±SEM) sperm counts of monkeys before (three or four samples) and at 2-week intervals during 44 weeks of Ag administration and during a 20-week recovery (R) period. *Top*, Group 1 animals given 25 μg/day Ag; *middle*, group 2 animals given 25 μg/day Ag and T; *bottom*, group 3 animals given 5 μg/day Ag for 24 weeks and then 10 μg/day Ag for 20 weeks.

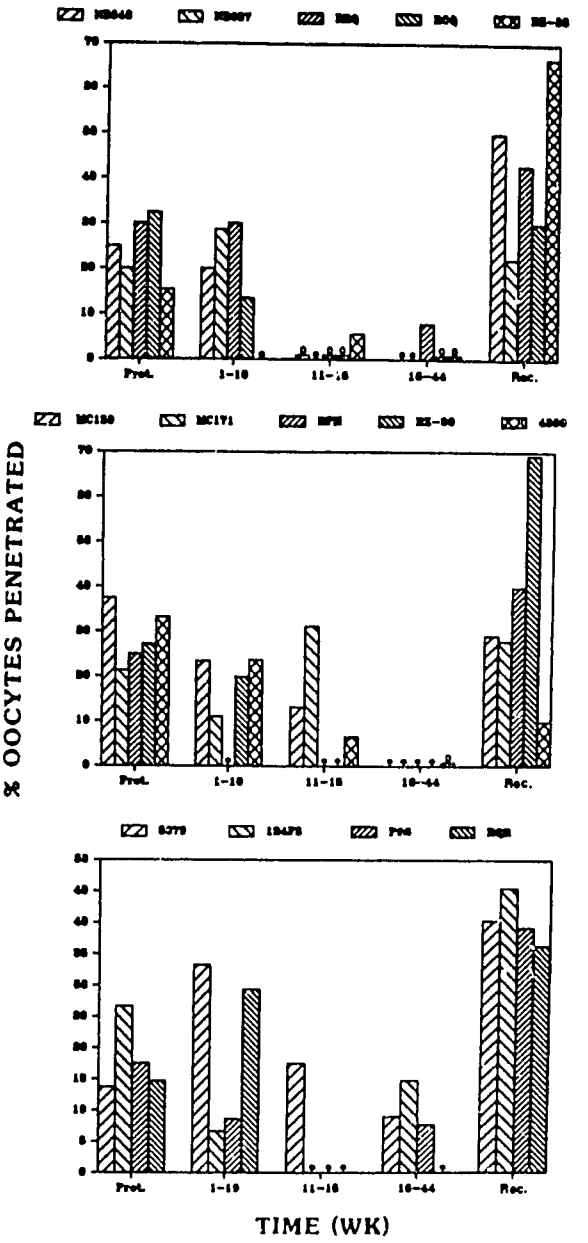

FIG. 5. SPA for individual monkeys during the control period (Pret.), at intervals during the administration of 25 μg/day Ag (group 1; *top*), 25 μg/day Ag and T (group 2; *middle*), or 5–10 μg/day Ag (group 3; *bottom*), and at 20 weeks of the recovery (Rec.) period. O, No oocytes penetrated. *, No sample available on the day of the test.

key to monkey, their seminiferous tubules contained Sertoli cells and, in most cases, a few identifiable spermatogonia and spermatocytes, but no early or late stage spermatids. No significant peritubular fibrosis or calcification was observed. Morphologically, testicular biopsies from groups 1 and 2 were indistinguishable (Fig. 7). Testicular histology from the one monkey in group 1 who did not become azoospermic revealed normal tubular cellularity and active spermatogenesis with progression to the late spermatid stage in almost all tubules.

Animals in group 3 had a less severe suppression of spermatogenesis. Tubules from these animals had reduced levels of spermatogenesis, with the most prominent stages being spermatocytes and early spermatids, and only a few late stage spermatids. One animal in this group had suppression of spermatogenesis comparable to that in monkeys in groups 1 and 2.

In all groups, there was severe atrophy of the seminiferous tubules. Tubular diameters declined 46% ($P < 0.001$) in group 1, 53% ($P < 0.001$) in group 2, and 35% ($P < 0.025$) in group 3.

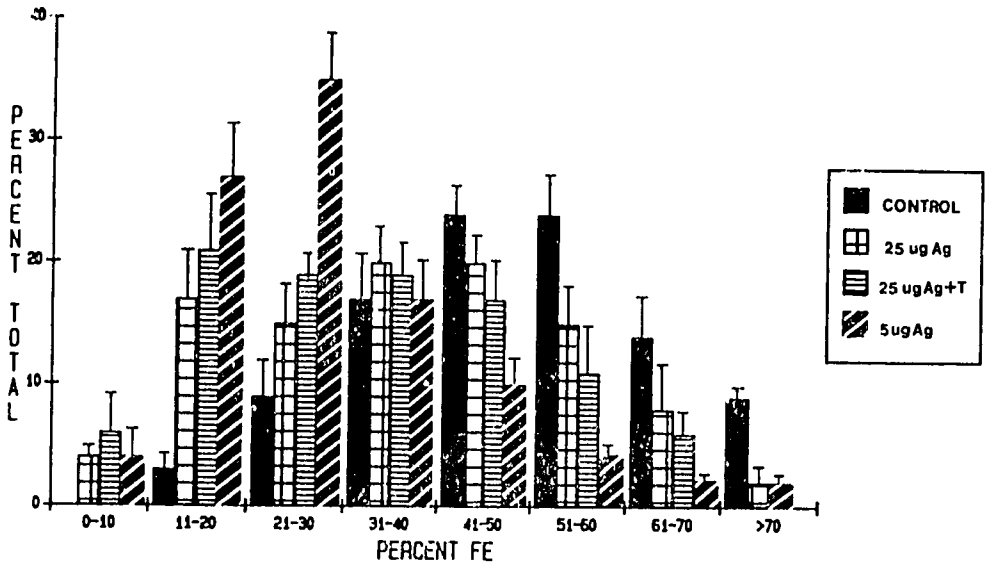

FIG. 6. Mean (±SEM) net negative surface charge on sperm measured indirectly subsequently to the attachment of positively charged colloidal iron to the surface of sperm. The data are presented as the percentage of iron relative to the other elements on the sperm surface *vs.* the percentage total of sperm counted (100/sample). The surface charge on sperm was measured on 17 samples from 9 monkeys during the control period and again on 26 samples between 5 and 10 weeks of administration of 25 μg/day Ag (group 1; n = 11 samples from 3 monkeys), 25 μg/day Ag and T (group 2; n = 8 samples from 3 monkeys), and 5 μg/day Ag (group 3; n = 7 samples from 3 monkeys).

Body weight

Body weights did not change in any of the three treatment groups.

Discussion

The results of this study clearly show that azoospermia can be induced in rhesus monkeys treated with 25 μg/day Ag and simultaneously given T to maintain the ejaculatory response. T administration did not attenuate the response to the analog, as reported previously (3, 4). Monkeys treated with Ag and T exhibited desensitization of the pituitary to GnRH and a decline in serum LH values to near-minimal levels of detection as rapidly as animals treated with Ag alone. Moreover, the rate of decrease and the lower testicular volume achieved during Ag treatment were not affected by T administration. Azoospermia occurred in all animals treated with Ag by 16 weeks (mean, 12.6). It was not possible to compare the time of onset of azoospermia with that in animals treated only with 25 μg/day Ag, because two of the four monkeys that became azoospermic in the latter group also had a decreased sensitivity to electroejaculation before their first azoospermic ejaculate. However, all four animals in this group were azoospermic by 21 weeks of treatment, suggesting that the times of onset of azoospermia were similar in animals treated with 25 μg/day Ag alone or with T. Thus, our data do not support the conclusion of other investigators (3, 4) that the administration of physiological doses of T to maintain libido and potency interferes with the induction of azoospermia with a GnRH analog.

The reason for the discrepancy between these and previous results in monkeys (3, 4) is not readily apparent. Ag administration was terminated at 22 weeks in the previous study, so azoospermia might have been achieved if the treatment period had been prolonged. However, all five monkeys treated with Ag and T in our study were azoospermic by 16 weeks. T administration reinitiates spermatogenesis in pituitary stalk-sectioned monkeys and stimulates spermatogenesis in immature monkeys (13, 14). However, the circulating T levels needed to initiate spermatogenesis were 10- to 20-fold higher than those found to maintain the ejaculatory response in our study and the previous report (3). Moreover, circulating T concentrations were comparable in the two studies (3). Thus, differences in maintenance doses of T may not account for the conflicting results. One major difference between the two studies is the use of different Ags.

One of the monkeys treated with 25 μg/day Ag alone never became azoospermic. Moreover, the pituitary of this animal continued to show significant, although reduced, serum LH and T responses to GnRH. We have treated 18 male monkeys with 25 μg/day Ag; this is the first animal that had a reduced response to this analog. In fact, 4 of 5 monkeys treated with first 5 then 10 μg/day Ag in this study had a number of azoospermic ejaculates during the late treatment and early recovery periods. We have no explanation for the apparent insensitivity of this 1 animal to the analog. It is unlikely that minipump malfunction explains the failure of this monkey to respond, since residual volumes of Ag remaining when the minipumps were removed did not vary significantly from pump to pump.

Earlier data from clinical trials in humans suggested that it might not be possible to induce azoospermia with Ag (5, 6, 15, 16). However, these studies were of limited duration (10–20 weeks), and except for the third study, the agonist was administered by daily sc injection, a method less effective than continuous administration (4, 17). In all of these studies, oligospermia, but not azoospermia, was achieved. Two recent reports (18, 19) offer

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FIG. 7. Testicular histology in male rhesus monkeys. A, Before Ag administration (×200); B, after 44 weeks of 25 μg/day Ag (×200); C, after 44 weeks of simultaneous treatment with 25 μg/day Ag and T (×200).

more hope for this contraceptive approach. Bouchard *et al.* (18) treated two groups of five men each with the D-Trp^6-GnRH agonist injected sc in biodegradable microspheres that released 100 μg agonist/day. One group received low dose androgen replacement sufficient to maintain serum T levels in the 1–3 ng/mL (3.5–10.4 nmol/L) range. A second group received a higher dose that maintained T values in the 2–5 ng/mL (6.9–17.3 nmol/L) range. Androgen therapy was begun 40 days after the beginning of agonist treatment. Four of the five men in the first group (low dose of T) became azoospermic by 28 weeks, while azoospermia was not achieved in the second group even though treatment was continued for 35 weeks. Swerdloff *et al.* (19) found that 400 μg/day of an agonist given by constant infusion plus 200 mg T enanthate given by im injection every 2 weeks resulted in azoospermia in three of seven men. These results suggest that azoospermia can be achieved in men with GnRH analog administration even if androgen replacement is provided to maintain libido. The dose of androgen may be important in determining whether azoospermia can be obtained while maintaining libido and potency.

One of the monkeys in our study had refractiveness to electroejaculation despite T replacement during the late treatment period (weeks 27–44). We have previously shown that continuous administration of Ag in monkeys is not only associated with a reduction of basal T secretion, but also abolishes the nocturnal rise in serum T (17). The maintenance dose of T used in this study was based on mean morning serum T levels during the pretreatment period. These levels may be less than the mean 24-h value and may not be sufficient to maintain the ejaculatory response. The critical aspect of T in maintaining spermatogenesis in primates and rodents is the intratesticular concentration (13, 14, 20–22). While as little as 10–20% of the normal intratesticular T concentration will maintain or initiate spermatogenesis (21, 22) in stalk-sectioned hypophysectomized adult or immature monkeys, T administration sufficient to raise serum concentrations 5- to 10-fold higher than normal adult male levels appear necessary to initiate qualitatively normal, but quantitatively reduced, spermatogenesis (13, 14).

There was a significant reduction in semen quality before the onset of azoospermia in Ag-treated monkeys. The percentages of motile and live sperm were significantly reduced in ejaculates with subnormal sperm counts. Moreover, the net negative surface charge on sperm, an indication of sperm maturation and potential fertilizing capacity (23), was reduced by Ag infusion. Sperm samples from individual monkeys treated with 25 μg/day Ag also had a reduced ability to penetrate hamster oocytes with longer Ag treatment periods. Importantly, T administration did not improve any of these indicators of semen quality. Thus, it was possible to maintain the ejaculatory response with T replacement without increasing the potential fertilizing capacity of the semen.

Sperm undergo significant changes during development and maturation within the testes and epididymis. Many of these changes occur on the sperm surface and are related to the acquisition of fertilizing capacity (24–

27). There is an increase in the net surface charge of sperm as they mature in the epididymis and a further increase at the time of ejaculation (9, 23, 28). A reduced surface negative charge of spermatozoa may be associated with reduced fertilizing capacity (23). These data coupled with results showing a decline in the ability to penetrate hamster oocytes suggest a significant decline in the potential fertilizing capacity of sperm from the oligospermic male treated with Ag.

Based on the work of Tezon *et al.* (29), a change in the surface composition or surface charge of primate sperm would be expected in monkeys treated only with Ag that are steroid deprived, but not with combined Ag and T administration. This hypothesis is not supported by our data, since a shift to a reduced net negative charge on sperm was observed in monkeys treated with Ag alone and Ag and T. However, if the alteration of surface charge reflects reduced steroid hormone levels in the epididymis, the level of T replacement did not provide adequate T levels to the epididymis. Alternatively, a steroid hormone other than T is the active steroid hormone in the epididymis. Another explanation is that the reduced gonadotropin levels affect sperm maturation either directly or indirectly.

The validity of the SPA as a measure of fertilizing capacity is controversial (30, 31). However, the test does monitor a significant aspect of sperm function. Ausmanas and collaborators (30) reported good correlation between the SPA and *in vitro* fertilization of human oocytes, and Rogers (32) found good correlation between this test and the motility and morphology of sperm. The changes in the SPA test with Ag treatment and alterations in motility and sperm surface charge, taken together, suggest that the potential fertilizing capacity of sperm from Ag-treated monkeys is significantly impaired before the onset of azoospermia.

The effects of Ag treatment on testicular histology are dramatic, but reversible. As we reported previously (2), seminiferous tubules from Ag-treated monkeys that become azoospermic generally contain only Sertoli cells and a few spermatogonia and spermatocytes. No later stages of spermatogenesis were evident. T supplementation did not protect the seminiferous tubules from the effects of the Ag. The atrophy of the tubules, the resultant decrease in testicular volume, and the suppression of spermatogenesis were not influenced by T levels sufficient to maintain the ejaculatory response.

Acknowledgments

We thank Wyeth Laboratories for providing the Ag used in this study, and the NIDDK for providing the rhesus monkey pituitary reference preparation (LER-1909-2) used in the LH bioassay. The skilled technical assistance of Ms. Janice Tittle and Mr. Thomas Buckner is gratefully acknowledged.

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Vol. 68, No. 3
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Vol. 68, No. 3
Printed in U.S.A.

Blockade of Neonatal Activation of the Pituitary-Testicular Axis: Effect on Peripubertal Luteinizing Hormone and Testosterone Secretion and on Testicular Development in Male Monkeys*

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ABSTRACT. The objective of this study was to examine the effect of blockade of neonatal activation of the pituitary-testicular axis, using a GnRH agonist, on sexual development in male rhesus monkeys. Monkeys were treated with either a GnRH agonist (10 μg/day; n = 8) or vehicle (n = 9) for 112 days using osmotic minipumps beginning at 10–13 days of age. In control monkeys serum LH and testosterone concentrations during the first 3 postnatal months were similar to those in adults; they then declined to very low levels. GnRH agonist administration caused an immediate and precipitous decline in serum LH and testosterone concentrations to very low levels, and both remained low throughout the rest of the agonist administration period. Neither group had any significant elevation in serum LH or testosterone concentrations during the next 2 yr. In the control monkeys serum LH and testosterone began to rise during the third year, with a rapid increase occurring during the fall coincident with the breeding season. This peripubertal rise of LH and testosterone secretion was associated with rapid enlargement of the testes and the appearance of sperm in ejaculates. The monkeys who had received GnRH agonist had subnormal serum LH and testosterone increases, and testicular enlargement was also attenuated compared to that in the control animals during the third year of life. Semen samples were recovered from only 50% of the GnRH agonist-treated monkeys during this period, and the sperm count per ejaculate was suppressed. The serum LH responses of the GnRH agonist-treated monkeys to an iv bolus dose of GnRH (5 μg/kg EW) during the third year were normal. These results suggest that the induction of reversible hypogonadotropin-hypogonadism in neonatal male monkeys alters subsequent testicular development and peripubertal endocrine changes. Thus, neonatal activation of the pituitary-testicular axis may be a critical developmental event in the process of sexual development in male primates. (*J Clin Endocrinol Metab* 68: 0000, 1989)

THE EARLY postnatal period of development is associated with an active period of LH and testosterone secretion in humans (1–4) and other primates (5–12). Serum LH, FSH, and testosterone concentrations are elevated for the first 3–6 months of postnatal life before falling to low basal levels where they remain until the time of puberty. The significance of the elevated gonadotropin and testosterone secretion during this period to subsequent sexual maturation of the male is unknown, and the consequences of abnormal pituitary-gonadal function at this time are uncertain. Neonatal human males with cryptorchidism have been reported to have reduced (13, 14), normal (15), or elevated (16) serum testosterone levels. Similarly, the gonadotropin responses of cryptorchid males to GnRH have been reported to be subnormal (17) or normal (18). While there may be no general agreement as to whether the hypothalamic-pituitary-testicular axis is functioning normally during the neonatal period in these patients, reduced testosterone production during this period may be responsible for the high frequency of defects in the seminiferous tubules of cryptorchid children and the high incidence of oligospermia in men treated as children for cryptorchidism (19).

Several years ago we reported that it was possible to block neonatal activation of the pituitary-testicular axis in male rhesus monkeys by continuous infusion of a GnRH agonist (20). In this study, we describe the effect of this blockade on subsequent changes in LH and testosterone secretion and on the process of testicular de-

Received August 25, 1988.

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* This work was supported by NIH Grants RR-08248 (via cofunding from the NIMH), RR-00165, and HD-23295; NSF Grant BNS 83-07295; and USAID Grant DAN-5053-G-SS-5018-00.

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velopment from the postnatal period until the onset of puberty. The objective was to determine whether the neonatal rise in testosterone was critical for normal sexual development in male primates.

Materials and Methods

Ten male rhesus monkeys (*Macaca mulatta*) that were born in 1984 to a heterosexual group of approximately 75 monkeys were studied. Six monkeys were treated continuously with 10 μg/day GnRH agonist (D-Trp^6-*N*-α-Me-Leu^7-des-Gly^{10}-Pro^9-NHEt-GnRH; Wyeth Laboratories, Philadelphia, PA), and 4 received vehicle (sterile water) from 10–122 days of age using osmotic minipumps (2 mL; Alza Corp., Palo Alto, CA). GnRH agonist or vehicle treatment was initiated at 10–13 days of age rather than at birth to allow a recovery period after parturition for both the mother and infant. The infants were kept with their mothers within the social group in a large outdoor compound (30 × 30 m) with attached indoor quarters during the first 3 yr of life. Then, all 10 monkeys were moved to another outdoor compound (15 × 15 m) containing 8 ovariectomized female monkeys in order to facilitate more extensive monitoring during the peripubertal period.

An additional group of 10 infants that were born in the same colony during 1983 also were studied. Five of these monkeys were given GnRH agonist for 112 days, as described above. The other 5 monkeys served as controls [only 2 of these monkeys were subjected to surgical procedures (pump implanted and removed)]. We reported previously the effect of GnRH agonist administration on serum LH and testosterone levels and on the response to GnRH during the neonatal period in this group of monkeys (20). In the 1983 birth cohort there were no detectable differences in body weight gain or crown-rump length during infancy between control monkeys subjected to the surgical protocols and those that were not or between control and agonist-treated monkeys (20). Thus, surgical procedures *per se* did not alter development during infancy. The 1983-born males were removed from the heterosexual group at 3 yr of age and kept indoors in controlled lighting (12 h of light and 12 h of dark) and temperature (22–25 C) conditions in single animal caging. Of the 5 animals treated with the agonist in 1983, only 2 animals are still alive, and only their data are included. One of the animals died of yersiniosis at approximately 1 yr of age, a second was killed at 2 yr of age because of inoperable laryngeal stenosis, and a third was killed at 3 yr of age because of repeated irreducible rectal prolapse. There was no evidence that these medical problems were related to GnRH agonist administration during infancy.

Treatment procedures

Minipumps were implanted sc under sterile conditions in ketamine-anesthetized (20–30 mg/kg) infants. The pumps were normally replaced every 4 weeks using similar sterile procedures. In certain monkeys, the pumps had to be replaced earlier because of infection or pump failure. In such monkeys we were unable to detect a significant effect on serum LH and testosterone concentrations.

Blood samples (1 mL; 1000–1400 h) were taken from unsedated monkeys before and after 4 and 10 days of GnRH agonist or vehicle administration and thereafter twice monthly for the remainder of the treatment period. After the monkeys reached 4 months of age the frequency of blood sampling was reduced to 1/month, and the volume of blood taken was increased to 3 mL/sample. Beginning at 3 yr of age, testicular volume was determined at monthly intervals, and the monkeys were subjected monthly to rectal probe electroejaculation under ketamine anesthesia, as previously described (21). When we were successful in collecting a semen sample; the sperm count and the percentages of motile and live sperm were determined. At yearly intervals beginning at 1 yr of age, each 1984-born monkey received an iv bolus dose of 5 μg/kg BW weight GnRH. Blood samples were taken before and 30 and 60 min after GnRH administration. Serum samples were stored at −20 C until assayed for LH and testosterone.

Hormonal assays were performed at intervals throughout the study. Serum LH was assayed in duplicate using the mouse interstitial cell-testosterone bioassay as originally described by Van Damme *et al.* (22) and as modified by Steiner and Bremner (8). The sensitivity of the LH assay was 0.2 mg/L, and the usable range of the assay was from 0.2–10 mg/L. The intra- and interassay coefficients of variation were 8.1% and 19.5%, respectively. The LH results are expressed in terms of the rhesus monkey pituitary gonadotropin standard, LER-1909-2, which has a potency of 0.0032 × NIH LH S1. Serum testosterone concentrations were measured in duplicate by RIA, as reported previously (23). The intra-assay and interassay coefficients of variation were 5.0% and 8.2%, respectively, and the sensitivity of the assay was 1.04 nmol/L.

Hormone levels that were undetectable were assigned values corresponding to the sensitivity limit of the assay (0.2 mg/L for LH; 1.04 nmol/L for testosterone). The hormonal data were initially analyzed using a two-way analysis (treatment × age) of variance, followed by a least significant difference test for between-group differences or a Tukey test for multiple comparisons within groups (24). The testicular volume data were first subjected to Bartlett's test for homogeneity of variance. The test indicated that the variance in the data between groups was not homogeneous. As a result, raw data were subjected to a square root transformation before two-way analysis of variance. $P < 0.05$ was considered significant.

Results

The mean serum LH concentrations in the 1984-born control monkeys were elevated for the first 4 months of postnatal life. They then declined to near the level of detection (0.2 mg/L; Fig. 1, *top*). The mean serum LH levels in the GnRH agonist-treated monkeys fell rapidly from a pretreatment value of 3.1 ± 1.5 (±SE) mg/L to less than 1.0 mg/L after 2 weeks of treatment and were lower ($P < 0.05$) than those in the control monkeys on most occasions during the first 3 months of postnatal life (Fig. 1, *top*).

The changes in serum LH concentrations were paralleled by comparable changes in serum testosterone levels (Fig. 1, *bottom*). A two-way analysis of variance indicated

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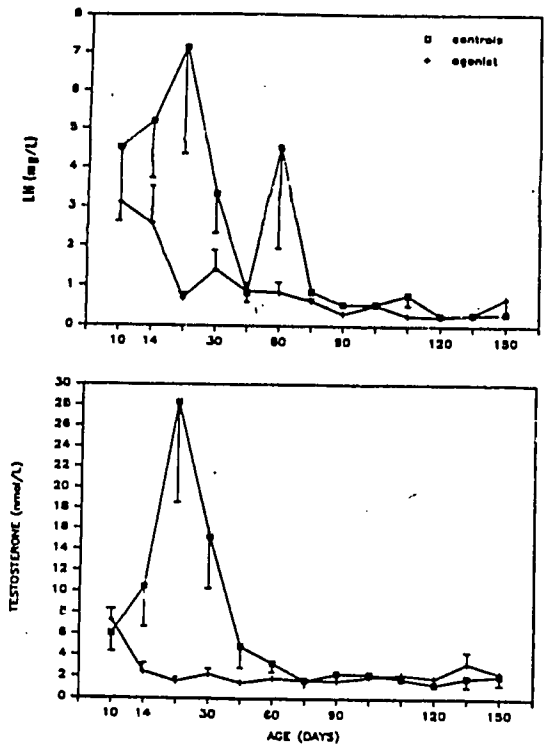

FIG. 1. Effect of GnRH agonist administration on mean (±SE) serum LH (*top*) and testosterone (*bottom*) concentrations in infant male rhesus monkeys. Control, n = 4; agonist-treated, n = 6. The animals were born in 1984. The scale of the x-axis has been expanded during the early neonatal period for clarity and to accommodate the additional blood samples taken during this period.

that there was a significant effect of GnRH agonist administration ($P < 0.025$) and age ($P < 0.01$), and a significant interaction ($P < 0.001$) between these two variables on serum testosterone levels. In the control monkeys, the mean serum testosterone concentration increased from 6.0 ± 2.0 nmol/L at 10–13 days of age to a peak of 28.4 ± 10.9 nmol/L at 3 weeks of age; it then gradually declined to less than 2 nmol/L by 75 days. GnRH agonist treatment caused an immediate decrease in mean serum testosterone values from 7.3 ± 1.5 nmol/L to undetectable values after 2 weeks, and they remained low for the remainder of treatment. Thus, GnRH agonist administration abolished the active period of LH and testosterone secretion characteristic of the neonatal period in monkeys. The changes in serum LH and testosterone concentrations as a result of neonatal GnRH agonist administration reported here for 1984-born monkeys are comparable to those reported earlier (20) for monkeys born in 1983.

GnRH agonist administration did not alter early postnatal growth in the 1984-born monkeys. There were no differences in body weight gain and crown-rump length between the control and agonist-treated monkeys during the first 20 weeks of postnatal life (Table 1).

By the time GnRH agonist administration was terminated between 122–125 days of age, the serum LH and testosterone values in the control and GnRH agonist-treated monkeys were similar (Fig 1). Thereafter, the mean serum LH and testosterone concentrations remained near the minimum levels of detection in both groups between 0.5–2.0 yr of age (data not shown).

The mean serum LH and testosterone concentrations in the 1984-born control and GnRH agonist-treated monkeys between 2 and 4 yr of age are shown in Figs. 2 and 3 (*top panels*). There were no differences in serum LH and testosterone concentrations between the two groups from 2 until 3 yr of age. In the control monkeys serum LH concentrations began to rise just before 3 yr of age (January 1987), and from January until September of that year they averaged about 1.0 mg/L. These values were significantly above the limit of detection, but were still below adult values. Beginning in October 1987, serum LH concentrations increased rapidly, coincidous with the breeding season, to a peak level of 4.1 ± 0.2 mg/L in November, and then declined to 0.5 ± 0.05 mg/L by April of 1988. This same pattern of serum LH concentrations was found in the GnRH-treated monkeys during the third year of life; the values in these monkeys were slightly but not significantly lower than those in the control monkeys. The serum LH concentrations also were slightly lower in the two 1983-born GnRH agonist-treated monkeys compared to those in the 3-yr-old age-matched control monkeys (Fig. 2, *bottom*).

The patterns of serum testosterone values during the third year of life in the 1984-born monkeys were similar to those of serum LH. Quantitatively, the differences in serum testosterone concentrations between the control and the GnRH agonist-treated monkeys were much greater (Fig. 3, *top*). In the control monkeys the mean serum testosterone levels rose from 4.9 ± 0.6 (±SE) nmol/L in September 1987 to greater than 15 nmol/L in October, November, and December 1987 before declining to 5.7 ± 0.9 nmol/L in January 88. During this same period, the mean serum testosterone levels in the GnRH agonist-treated monkeys never exceeded 5.5 nmol/L (effect of agonist treatment; $P < 0.01$). The differences in serum testosterone concentrations were comparable between the GnRH agonist-treated and control monkeys born in 1983 (Fig. 3, *bottom*).

The mean testicular volume was lower ($P < 0.05$) in the 1984-born GnRH agonist-treated monkeys compared to that in the control monkeys during the third year of life (September through May; Fig. 4, *top*). The GnRH

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TABLE 1. Body weights and crown-rump (CR) lengths in control (n = 4) and GnRH agonist-treated (n = 6) male rhesus monkeys born in 1984

	Treatment week					
	0	4	8	12	16	20
BW (kg)						
Control	0.55 ± 0.04	0.65 ± 0.06	0.85 ± 0.05	1.02 ± 0.06	1.21 ± 0.10	1.21 ± 0.04
Agonist	0.54 ± 0.03	0.72 ± 0.03	0.81 ± 0.04	0.91 ± 0.05	1.13 ± 0.05	1.25 ± 0.05
CR (cm)						
Control	21.1 ± 0.6	22.6 ± 0.8	24.1 ± 0.7	25.5 ± 0.6	26.6 ± 0.4	27.6 ± 0.3
Agonist	20.8 ± 0.5	22.7 ± 0.3	23.4 ± 0.7	24.9 ± 0.5	26.4 ± 0.4	26.9 ± 0.3

Values are the mean ± SE. GnRH agonist treatment was begun between 10 and 13 days of age and terminated between 122–125 days of age.

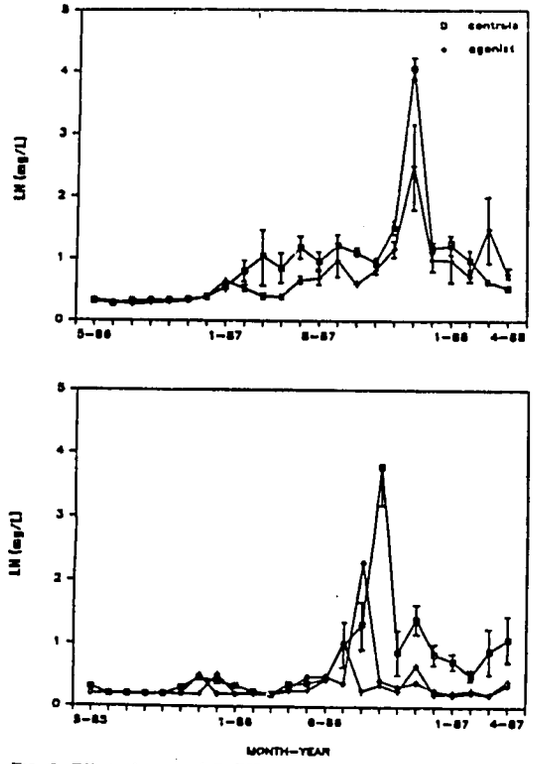

FIG. 2. Effect of neonatal GnRH agonist administration on mean (± SE) serum LH concentrations in rhesus monkeys from 2–4 yr of age. The animals in the *top graph* were born in 1984 (control, n = 4; agonist-treated, n = 6); those in the *bottom graph* were born in 1983 (control, n = 5; agonist-treated, n = 2). The individual values for the two agonist-treated monkeys born in 1983 are shown in the *bottom graph*.

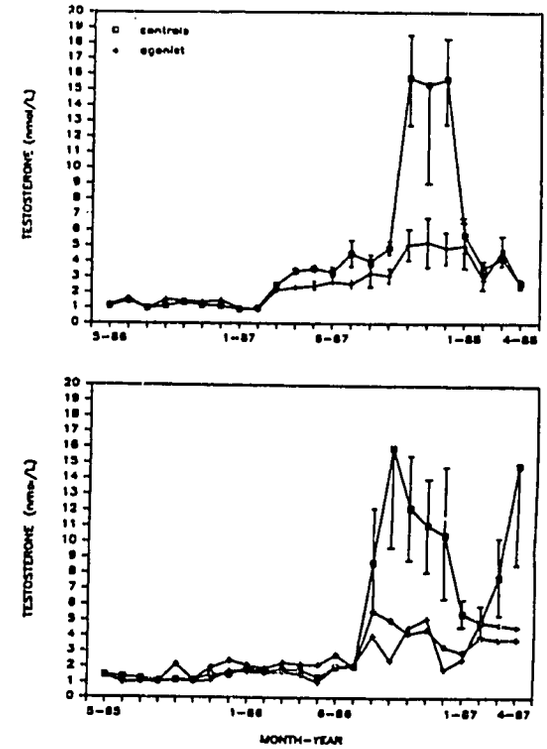

FIG. 3. Effect of neonatal GnRH agonist administration on mean (± SE) serum testosterone concentrations in rhesus monkeys from 2–4 yr of age. The animals in the *top graph* were born in 1984 (control, n = 4; agonist-treated, n = 6); those in the *bottom graph* were born in 1983 (control, n = 5; agonist-treated, n = 2). The individual values for the two agonist-treated monkeys born in 1983 are shown in the *bottom graph*.

agonist-treated monkeys born in 1983 also had smaller testes than the control monkeys during their third year (Fig. 4, *bottom*).

We were able to collect semen samples by electroejaculation from all 4 control monkeys born in 1984 [13 ejaculates/16 attempts (81%)] but from only 3 of the 6 GnRH agonist-treated animals [6 ejaculates/20 attempts (30%)] during the fall and winter of their third year. The sperm counts in 3 of the control monkeys were in the low normal to normal adult range (range, 20–2500 × 10^6/mL; mean ± SE, 532 ± 276 × 10^6/mL). We were able to collect only 2 samples from the fourth control monkey; both samples had low sperm counts (2–3 × 10^6/mL). The mean percent motile and percent live sperm/ejaculate

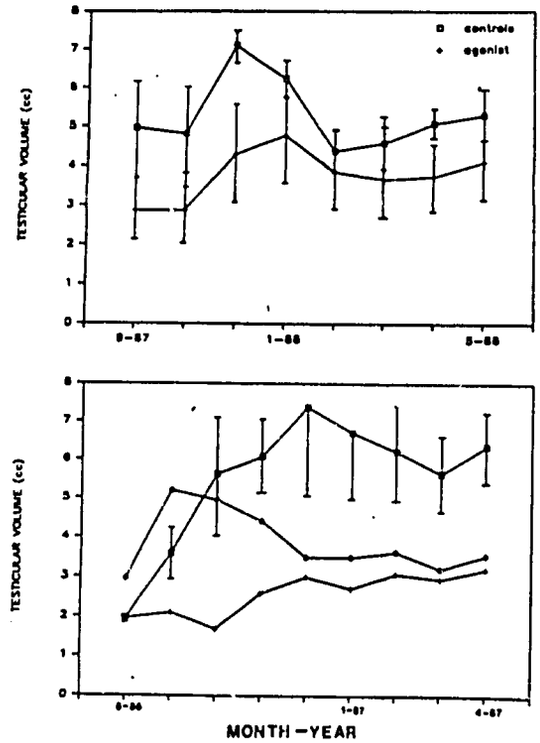

FIG. 4. Effect of neonatal GnRH agonist administration on mean (± SE) testicular volumes in rhesus monkeys during the third year of life. Animals in the *top graph* were born in 1984 (control, n = 4; agonist-treated, n = 6); those in the *bottom graph* were born in 1983 (control, n = 5; agonist-treated, n = 2). The individual values for the two agonist-treated monkeys born in 1983 are shown in the *bottom graph*.

were 65 ± 7% (±SE) and 73 ± 3%, respectively, for the 4 control monkeys. In the 3 GnRH agonist-treated monkeys who had an ejaculatory response, the sperm counts were low (1–67 × 10^6/mL; mean ± SE, 42.2 ± 27.6 × 10^6/mL), and the percent motile and percent live sperm per ejaculate values were comparable to those in the control monkeys.

The six monkeys born in 1984 who received GnRH agonist clearly fell into two separate groups, based both on their serum testosterone levels and whether we were able to collect semen samples from them during their third year of life (Fig. 5). A comparison of the serum testosterone data between the GnRH agonist-treated monkeys from which we were either able or unable to collect sperm revealed a significant interaction ($P < 0.025$) of the two variables (semen sample *vs.* no semen sample × time). Three monkeys had a subnormal but significant peripubertal rise in semen testosterone concentrations, and it was from the same monkeys that we

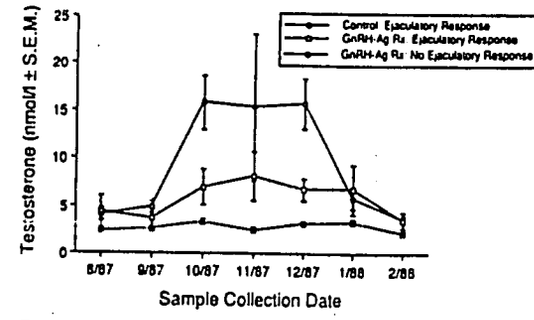

FIG. 5. Mean (±SE) serum testosterone concentrations in control and agonist-treated monkeys during the fall months of the third year of life. The animals were born in 1984. The agonist-treated monkeys were separated into two groups of three monkeys each according to whether we were able to collect semen samples from them.

were able to collect semen samples. The other three monkeys had neither a significant rise in serum testosterone concentrations nor an ejaculatory response during this period. The reduced serum testosterone levels in these three monkeys was probably a result of lower ($P < 0.025$) serum LH concentrations (data not shown).

The responses of 1984-born monkeys to an iv bolus dose of GnRH at 1, 2, and 3 yr of age are shown in Fig. 6. There were no differences in the serum LH responses to GnRH between the control and GnRH agonist-treated animals at any age, and the LH responses to GnRH increased with age in both groups (Fig. 6, *top*). The serum testosterone values both before and after GnRH treatments were reduced ($P < 0.05$) in the GnRH agonist-treated monkeys compared to those in the control monkeys at 3 yr of age (Fig. 6, *bottom*), but the incremental responses were similar. The serum testosterone responses to GnRH also were very similar in the GnRH agonist-treated and control monkeys at 1 or 2 yr. In both groups of monkeys the serum testosterone responses to GnRH increased with increasing age.

Neonatal GnRH agonist administration did not significantly affect body weight in monkeys between 10 days and 4 yr of age, although the tendency was for body weight to be reduced in the GnRH-agonist-treated monkeys between 2 and 4 yr of age (Table 2).

Discussion

These results suggest that the early postnatal period is a critical period in the maturation of the hypothalamic-pituitary-gonadal axis in male primates. During the first 3–5 months of life, there is activation of the hypothalamic-pituitary-testicular axis (1–12). We found that reversible inactivation of the pituitary and testes by GnRH agonist administration during the first 4 months of postnatal life in male monkeys retarded peripubertal testic-

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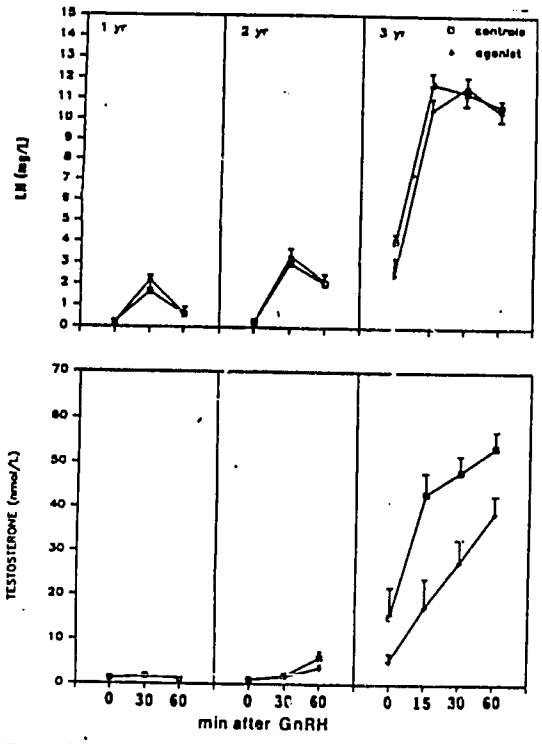

FIG. 6. Mean (±SE) serum LH (*top*) and testosterone (*bottom*) responses to an iv bolus dose of GnRH (5 μg/kg BW) in control and agonist-treated monkeys. The animals were given GnRH at 1, 2, and 3 yr of age. These animals were born in 1984. Control, n = 4; neonatal GnRH agonist-treated, n = 6.

ular development.

As we reported previously (20), GnRH agonist administration to neonatal male rhesus monkeys beginning soon after birth reduces serum LH and testosterone levels to very low levels, whereas serum LH and testosterone levels in control monkeys rise to adult values during the first 3 postnatal months. When GnRH agonist administration was terminated at 122–125 days of age, there was no rebound in either serum LH or testosterone. Thus, neonatal activation of the pituitary-testicular axis was not simply delayed, but was permanently prevented.

The decline in serum LH and testosterone levels during GnRH agonist administration in neonates was most likely due to a reduction of the pituitary response to GnRH. While we did not test pituitary responses to GnRH in the agonist-treated monkeys in this study, we reported previously (20) that male neonatal monkeys treated with a GnRH agonist did not have serum LH and testosterone responses to GnRH. Presumably, GnRH receptors in the neonatal pituitary, like those in the adult pituitary, are down-regulated by chronic GnRH agonist administration (25).

We found no differences in serum LH and testosterone levels between the control and GnRH agonist-treated monkeys from 6 months of age until early in the third year of life. During this period, serum LH and testosterone levels were low, characteristic of the juvenile phase of development (26), and the serum LH and testosterone responses to GnRH were minimal in both groups. Serum LH and testosterone levels began to rise during the third year in the control monkeys. The increase was small initially, but later accelerated with the approach of the breeding season in this species (27). In contrast, the serum testosterone rise increased considerably less during the third year in monkeys treated neonatally with the GnRH agonist. Although there was a tendency for serum LH levels to be lower in agonist-treated animals during the same period, these differences were not significant. The testicular volumes of these monkeys during the third year were smaller than those in the control monkeys. These results indicate that treatment with the neonatal GnRH agonist administration resulted in mild persistent hypogonadotropic hypogonadism.

Because blood samples were collected from the monkeys on a monthly basis, it is possible that differences in episodic LH and testosterone secretion between the control and the GnRH agonist-treated monkeys may have gone undetected. Since we were concerned that repeated experimental manipulation of the large social group (such as would be required for studying pulsatile LH and testosterone secretion) might confound or compromise our results, experiments of this type were not attempted.

The responses to GnRH during the third year provide further information concerning the retarded gonadal development in the GnRH agonist-treated monkeys. The serum LH responses in the GnRH agonist-treated and control monkeys were similar, as were the testosterone responses. These results suggest that neonatal GnRH

TABLE 2. Body weight in control and GnRH agonist-treated monkeys between 10 days and 4 yr of age

	Age						
	10 days	0.5 yr	1.0 yr	2.0 yr	3.0 yr	3.5 yr	4.0 yr
Control	0.55 ± 0.04	1.5 ± 0.1	2.7 ± 0.1	4.3 ± 0.1	6.3 ± 0.1	6.8 ± 0.3	7.4 ± 0.5
GnRH agonist	0.54 ± 0.03	1.3 ± 0.1	2.7 ± 0.1	4.0 ± 0.1	5.7 ± 0.3	6.2 ± 0.4	7.1 ± 0.6

Values are the mean ± SE.

agonist administration or the decreased LH and testosterone secretion that occurred in response to it altered development of the central nervous system centers governing episodic GnRH secretion, resulting in a delay in the onset of peripubertal episodic GnRH secretion and, consequently, in LH secretion and testicular development. Alternatively, LH secretion in response to endogenous GnRH was impaired, even though LH secretion in response to a pharmacological GnRH dose was not. A third possibility, that the GnRH agonist somehow altered testicular function directly, is not likely, since that might be expected to increase serum LH concentrations in the treated monkeys.

It is conceivable, but unlikely, that GnRH agonists have a direct effect on the central nervous system of neonates. In adult male and female monkeys, the inhibitory effects of GnRH agonists on gonadotropin secretion appear to be mediated entirely via down-regulation of pituitary GnRH receptors and postreceptor modification of LH secretion (28–30), and there is no evidence available to suggest that GnRH agonists alter GnRH secretion by the hypothalamus. Moreover, there is little evidence that GnRH analogs have a direct effect on the primate testis. The vast majority of studies, including several from our laboratory, indicate that in adult primates GnRH analogs do not alter testicular function directly (28, 31, 32). Thus, we believe it unlikely that the GnRH agonist had a direct effect on the development of either the central nervous system or testes. We, therefore, believe that its effect was on the pituitary, and the consequence was a poor pubertal increase in LH secretion.

It appears that the GnRH pulse generator is functional in the primate fetus (26). After birth, GnRH secretion and, therefore, LH secretion are episodic and circadian in primate males, and serum LH and testosterone levels reach adult levels (8, 10, 20). Moreover, bilateral orchidectomy of rhesus infants at 1 week of age causes a rise in LH and FSH secretion equal to that after castration in the adult, and LH secretion is pulsatile with a pulse frequency of one pulse per h in such animals (10, 26, 33). These data suggest that the hypothalamic-pituitary-testicular axis is fully mature at birth in male primates (26). However, this conclusion is primarily based upon data from male monkeys orchidectomized shortly after birth. Therefore, it is impossible to determine whether these monkeys would have a normal onset of puberty or normal peripubertal changes. The data from this study, using a GnRH agonist to induce reversible neonatal castration, suggest that the enhanced activity of the testis of neonatal primates may be a critical event in the developmental process that ultimately leads to sexual maturation in male primates.

It is also possible that neonatal testosterone secretion may influence not the central nervous system centers involved in the regulation of pituitary function, but, instead, those involved in the development of social and sexual behavior. The monkeys in this study were reared in a large social group for the first 3 years of life. Rose and collaborators (27) found that the peripubertal rise in serum testosterone levels in 3-yr-old adolescent male monkeys was inversely proportional to the number of adult males in the social group in which they were reared. This result may reflect the effect of the adult males on the rank of the adolescents in the social group (27). In this regard, the suppression of neonatal testosterone secretion that occurred in the GnRH agonist-treated monkeys may have influenced the interaction of these males with other members of the group, resulting in a suppression of testosterone secretion during the third year.

There appear to be parallels between neonatal GnRH agonist-treated male rhesus monkeys and human infants suffering from cryptorchidism as a result of early transient deficiency of testosterone secretion (19). This cryptorchid condition is caused by a primary deficiency of LH secretion which persists through childhood and early puberty and then disappears at midpuberty (19). It has been hypothesized that early postnatal deficiency in gonadotropin secretion and Leydig cell function contributes to impaired testicular descent and maturation (19). Adult men treated in childhood for cryptorchidism also have a high frequency of oligospermia (19). It is noteworthy that the three GnRH agonist-treated monkeys who had an ejaculatory response during the breeding season of their third year of life also had lower than expected sperm counts, although this conclusion is based on only six semen samples.

In summary, the induction of hypogonadotropic hypogonadism in neonatal male rhesus monkeys alters normal testicular development and peripubertal endocrine events. We do not yet know whether this early postnatal gonadotropin and testosterone deficiency has a permanent or only a temporary effect on pituitary-testicular function. Although these results are based on a limited number of monkeys and should not be overinterpreted, the consistency of the results across the two birth cohorts suggests that the early neonatal period may represent another period, in addition to the early prenatal period, when androgens exert an organizational influence (34) on the developing central nervous system of primates (35).

Acknowledgments

We thank Wyeth Laboratories for providing the GnRH agonist used in this study, the NIDDK for providing the rhesus monkey pituitary reference preparation (LER-1909-2) used in the LH bioassay, and Dr. Gordon Niswender for providing the antiserum for the testosterone

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RIA. We also thank Mr. Thomas Buckner, Ms. Toni Duffey, Ms. Robin King, and Ms. Janice Tittle for their expert technical assistance. The Yerkes Regional Primate Research Center is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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