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The gonadotropin-releasing hormone (GnRH) agonist-induced initial rise of bioactive LH and testosterone can be blunted in a dose-dependent manner by GnRH antagonist in the non-human primate*

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Summary. Gonadotropin-releasing hormone (GnRH) agonists induce a clinically undesirable, transitory but very pronounced initial rise of gonadotropin and gonadal steroid secretion. We investigated, in a non-human primate model, whether the initial stimulatory effects of GnRH agonists can be avoided by a short period of pretreatment and simultaneous treatment with a GnRH antagonist. Three groups of five adult male cynomolgus monkeys (*Macaca fascicularis*) received a single s.c. biodegradable implant loaded with the GnRH agonist, buserelin ([D-Ser(TBu)^6-desGly-NH_2]-GnRH), releasing approximately 50 μg buserelin daily. From 1 week before to 1 week after inception of administration of GnRH agonist, group 1 received the GnRH antagonist vehicle, and groups 2 and 3 were given s.c. injections of the GnRH antagonist Nal-Glu ([Ac-D-Nal(2)^1,D-4-Cl-Phe^2,D-Pal^3,D-Arg^5,D-Glu^6(AA),D-Ala^{10}]-GnRH) at a dose of 450 or 2250 μg/kg daily. In the absence of GnRH antagonist, the GnRH agonist induced a marked elevation of serum luteinizing hormone (LH) and testosterone lasting for 2 and 5 days, respectively. In group 2, Nal-Glu reduced basal hormone secretion and delayed the peak of GnRH-agonist-induced hormone secretion by 1 day. In group 3, the GnRH-agonist-induced rise of LH and testosterone was prevented in three animals and did not exceed baseline hormone levels in the other two animals. Areas under the LH and testosterone curves were significantly reduced in group 3 compared to group 1. After withdrawal of the GnRH antagonist, a second transient rise of hormone secretion was observed. Except for testosterone in group 2, this rise did not exceed the baseline range of hormone concentrations. The study demonstrates that high doses of GnRH antagonist can prevent the GnRH agonist-induced initial rise of LH and testosterone secretion, while after withdrawal of the GnRH antagonist a second rise of hormone secretion occurs, which is less pronounced than with GnRH agonist alone.

Key words: GnRH agonist – GnRH antagonist – Luteinizing hormone – Testosterone – Monkey

Secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus is required for the maintenance and regulation of pituitary and gonadal function. Agonists of GnRH initially cause a marked stimulation of gonadotropic and gonadal steroid hormone secretion lasting for several days in primates [17]. This elevation of hormone secretion is transient and the GnRH agonist subsequently down-regulates the pituitary GnRH receptor [11], leading to suppression of luteinizing hormone (LH) and release of gonadal steroid [1]. For this reason, GnRH agonists are widely employed for therapy of gonadal-steroid-related disease, i.e. precocious puberty, endometriosis, and prostate cancer [18], whereas their potential for male contraception has been questioned [2]. The transient initial rise of gonadal steroid hormones is clinically undesirable and has been found to be associated with aggravation of cancer-related symptoms and disease flare-up in prostate carcinoma patients [19, 23]. In patients with advanced prostatic carcinoma, antiandrogens have therefore been used to block androgen action during the initial stimulatory phase of GnRH-agonist therapy [23].

Unlike GnRH agonists, GnRH antagonists block the hypophyseal GnRH receptor [7] and suppress gonadotropin and gonadal steroid hormone secretion precipitously without any initial stimulation [24]. The histamine-releasing properties [10], other side effects [20] and the need for higher doses of GnRH antagonists than of GnRH agonists to obtain inhibitory effects have so far prevented the wide-spread clinical use of GnRH antagonists for therapeutic purposes. On the other hand, GnRH antago-

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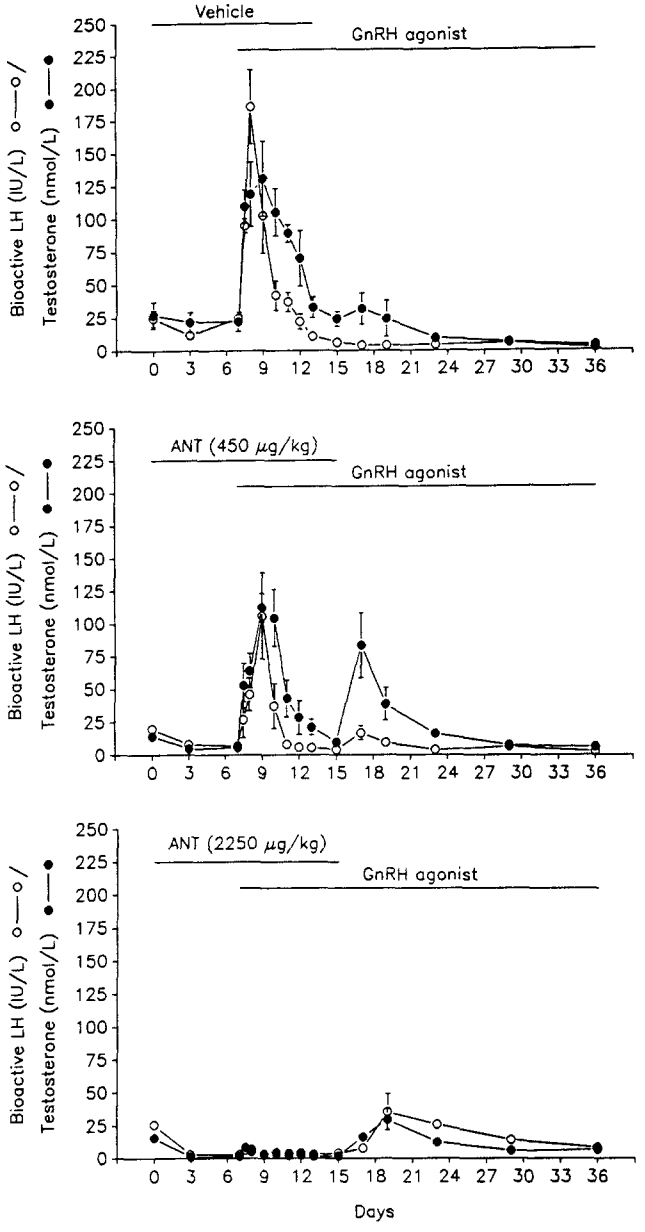

Fig. 1. Mean (±SE) of serum bioactive LH (open circles) and testosterone (filled circles) of adult male monkeys (five per group) receiving an implant loaded with GnRH agonist (buserelin) on day 7. On days 0–15 animals were treated, in addition to the GnRH agonist, with GnRH antagonist vehicle (upper panel) or Nal-Glu GnRH antagonist at a dose of 450 µg/kg daily (middle panel) or 2250 µg/kg daily (bottom panel). *ANT*, antagonist

nists are potent inhibitors of LH and testosterone secretion in normal men [3, 4, 9] and are promising candidates for male fertility regulation [8, 22].

Since GnRH agonists and antagonists compete for the same receptor at the pituitary level [28] the present study was undertaken to investigate whether a short period of pretreatment and simultaneous treatment with GnRH antagonist is capable of preventing the GnRH agonist-induced initial rise of LH and testosterone secretion in a male non-human primate model. A depot preparation GnRH agonist buserelin and the GnRH antagonist Nal-Glu, which has already been used clinically, were employed for this study.

Materials and methods

Animals and GnRH analogs

Fifteen adult male cynomolgus monkeys (*Macaca fascicularis*), weighing 4.1–6.5 kg, were caged individually in a room with controlled temperature, humidity and light (20 ± 1 °C; 40–45% relative humidity; lights on from 0700–2100 hours). Animals were fed twice daily with monkey pellets supplemented with fresh fruit. Water was available ad libitum. The studies were performed in accordance with the regulations of the German Federal Law on the Care and Use of Laboratory Animals.

The GnRH agonist buserelin [D-Ser(TBu)^6-desGly-NH_2]-GnRH was incorporated into biodegradable polylactic polyglycolide rods and one rod per animal was implanted subcutaneously [26]. The implants were designed to release approximately GnRH agonist 50 µg/daily and were effective for a 4-week period [25]. The GnRH antagonist Nal-Glu [(Ac-D-Nal(2)^1,D-4-Cl-Phe^2,D-Pal^3,Arg^5,D-Glu^6(AA),D-Ala^{10}-GnRH was dissolved in propylene glycol:saline (1:1, v/v) and injected s.c. [25].

Experimental design

Animals were divided into three groups of five monkeys each. Group 1 animals received daily s.c. injections of vehicle (1 ml) for 14 days. Animals in group 2 and group 3 received daily s.c. injections of GnRH antagonist 450 µg/kg and 2250 µg/kg respectively, for 14 days. In all animals, GnRH agonist rods were implanted on day 7 of treatment with vehicle or GnRH antagonist.

Blood samples were collected from the femoral vein under ketamine hydrochloride sedation (Ketavet, 10–15 mg/kg; Parke-Davis, Berlin, FRG) twice during baseline, on days 3, 7 (before and after 12 h of GnRH agonist implantation), 8, 9, 10, 11, 12, 13, 15, 17, 19, 23, 29 and 36 of the study. Serum was separated after clotting overnight at 4 °C by centrifuging twice in the cold for 20 min at 1000 g and stored at −20 °C until hormone analysis.

Hormone analysis

Serum concentrations of bioactive LH and testosterone were determined in all samples. Bioactive LH was measured in an in vitro mouse Leydig-cell bioassay as described previously [27]. The sensitivity of the assay was 2.6 IU LER 907/1. The intra- and interassay coefficients of variation were 14.6 and 15.5%, respectively. Testosterone was measured by readioimmunoassay in uncrhomatographed serum as reported previously [6]. The sensitivity of the assay was 0.8 nmol/l and the intra- and interassay coefficients of variation were 6.1% and 6.7%, respectively.

Evaluation of data

Data are presented as means ± SE. For values below the assay detection limits the detection limit was used for calculations. To assess the GnRH-agonist-induced stimulation of testosterone secretion, the area under curve during days 7–36 of the study (0–29 of GnRH-agonist treatment) was calculated by the trapezoid method [6]. Data for areas under curves were analysed by one-way analysis of variance. The multiple comparison test of Tukey was used to localize statistically significant differences following the analysis of variance procedure. The statistical computations were performed with the STSC Statistical Package (STSC, Rockville, Md., USA) at a significance level of $P < 0.05$.

Table 1. Response areas under bioactive LH and testosterone curves during days 0–8 of administration of GnRH agonist in adult male monkeys. Treatment with GnRH antagonist lasted from 7 days before until day 8 after administration of GnRH agonist (approximately 50 μg daily)

	Area under hormone curve (days 0–8)	
	Testosterone (nmol × d/l)	Bioactive LH (units × d/l)
GnRH agonist plus vehicle		
1	339	271
2	635	545
3	464	482
4	813	587
5	836	211
mean ± SE	617 ± 96	419 ± 75
GnRH agonist plus antagonist 450 μg/kg		
1	502	255
2	318	147
3	560	399
4	399	227
5	244	64
mean ± SE	405 ± 58	218 ± 56
GnRH agonist plus antagonist 2250 μg/kg		
1	18	24
2	18	34
3	39	39
4	8	24
5	34	31
mean ± SE	24 ± 5[a]	30 ± 3[a]

[a] $P < 0.01$ vs GnRH agonist plus vehicle group

Results

Basal hormone levels

Hormone concentrations of bioactive LH are depicted in Fig. 1. In group 1, which had not been exposed to GnRH antagonist, LH concentrations rose from 24.7 ± 4.9 to 94.9 ± 5.2 IU/l within 12 h after GnRH agonist treatment and peaked after another 12 h (185.8 ± 28.4 IU/l). Thereafter LH values steadily declined, became suppressed within 8–10 days after inception of GnRH agonist administration and remained low.

In group 2, receiving the lower dose of GnRH antagonist, LH concentrations were reduced from 19.7 ± 2.8 to 6.7 ± 0.2 IU/l at the time of inception of GnRH agonist treatment (7 days of pre-exposure to GnRH antagonist). After 12 and 24 h of treatment with GnRH agonist, LH levels were 26.7 ± 13.6 and 45.9 ± 12.3 IU/l, respectively, and peak concentrations were attained on day 2 (115.8 ± 45.1 IU/l). Within another 3 days, LH concentrations were lowered.

In group 3, given the higher dose of GnRH antagonist, concentrations of LH fell from 25.8 ± 4.8 to 2.7 ± 0.08 IU/l within the first 7 days of antagonist treatment with GnRH. The effect of GnRH agonist on LH secretion in this group was small and of short duration. After 12 and 24 h of GnRH-agonist treatment, LH values were 2.8 ± 0.1 and 6.4 ± 2.8 IU/l, respectively. On day 2, LH concentrations were 5.0 ± 1.5 IU/l and were fully suppressed again by day 3 (2.7 ± 0.06 IU/l). On an individual basis, only two animals exhibited a rise of bioactive LH and testosterone which did not exceed the baseline range.

Following withdrawal of GnRH antagonist, LH concentrations were elevated from 4.0 ± 0.2 IU/l to 16.7 ± 5.2 and 9.4 ± 2.5 IU/l after 3 and 5 days, respectively, in group 2. In group 3, values for LH were 3.8 ± 1.1 IU/l when GnRH antagonist was discontinued and rose to 7.4 ± 1.0 and 35.9 ± 13.7 IU/l 3 and 5 days later, respectively. By day 36 (29 days after initiation of GnRH agonist treatment) LH values were suppressed again.

A hormone pattern similar to that seen for LH was observed for testosterone (Fig. 1). In group 1, testosterone levels increased within 12 h from 21.9 ± 7.3 to 109.4 ± 12.7 nmol/l, and the highest concentrations (130.3 ± 28.5 nmol/l) were found on day 2. From days 16–22 of GnRH agonist administration onwards, testosterone values were lowered compared with baseline. In group 2, the 12 h value for testosterone was 52.8 ± 16.8 vs 5.5 ± 1.4 nmol/l and peak values were also seen after 2 days (112.1 ± 10.7 nmol/l). Decreased testosterone concentrations were found within 22 days. In group 3, GnRH antagonist treatment further suppressed testosterone levels (1.5 ± 0.3 nmol/l compared to 5.5 ± 1.4 nmol/l in group 2) and the GnRH agonist stimulated testosterone values to 8.4 ± 3.6 nmol/l (range: 1.2–19.4 nmol/l) within 12 h. After 24 h levels were 7.0 ± 2.7 nmol/l and after another day, testosterone concentrations were suppressed again.

The areas under bioactive LH and testosterone curves were calculated for the period 7–15 days of the study (0–8 days of GnRH agonist administration and period of concomitant exposure to GnRH agonist and antagonist). Data are given in Table 1. Areas under the bioactive LH curve were reduced to 52% ($P > 0.05$) in group 2, and to 7% ($P < 0.01$) in group 3 compared to group 1. Similarly, the areas under testosterone curves were reduced to 65.5% ($P > 0.05$) in group 2, but to 3.9% ($P < 0.01$) in group 3 compared with group 1.

Discussion

The present study evaluated the potential of a GnRH antagonist to prevent the initial stimulation of LH and testosterone secretion associated with GnRH agonist in a non-human primate model. In the animals subjected to treatment with GnRH agonist and GnRH antagonist vehicle, a highly pronounced initial rise of bioactive LH and testosterone secretion occurred within 12–24 h after initiation of GnRH agonist treatment. This initial stimulation of LH and testosterone secretion is characteristic of GnRH agonist treatment. The stimulatory effects on hormone secretion of the depot GnRH agonist preparation used in the present investigation have also been seen in male rhesus monkeys [26] and in normal men [2].

Pretreatment and simultaneous treatment with a GnRH antagonist for a period of 7 days each counteracted the GnRH-agonist-induced initial elevation LH and testosterone in a dose-dependent manner. At a dose of 450 μg/kg daily, the GnRH antagonist lowered, although not significantly, the bioactive LH and testosterone peak and delayed the onset of the inhibitory phase of GnRH-agonist-induced suppression of hormone release. However, the area under the testosterone curve was not significantly reduced when compared to the group without GnRH antagonist administration and, most importantly, the serum testosterone levels exceeded the normal baseline levels several-fold. On the other hand, a fivefold increase of the GnRH antagonist dosage prevented the GnRH-agonist-induced rise of bioactive LH and testosterone in three animals. In the remaining two monkeys, the GnRH-agonist-associated rise of both bioactive LH and testosterone was small and did not exceed the range of baseline concentrations.

These findings demonstrate that the initial transient supranormal secretion of LH and testosterone caused by the GnRH agonist can be avoided by treatment with high doses of a GnRH antagonist. It is established that both types of GnRH analogues bind to a common GnRH receptor in the pituitary cells [7, 28]. On these grounds, and based on the present observation that the counteractive effects of GnRH antagonist increased with a higher dose, it is assumed that an effective dose of GnRH antagonist must provide sufficiently high portal blood levels for successful competition with the GnRH agonist at the pituitary GnRH receptor. This view is corroborated by the previous demonstration in the non-human primate model that the inhibitory effects of a GnRH antagonist on bioactive LH and testosterone secretion could be overcome by exogenous GnRH stimulation in a dose-dependent fashion [13]. Alternatively, the GnRH-antagonist-induced suppression of LH secretion may not require complete blockade of pituitary GnRH-binding sites as shown in the rat [16]. In such case, the more pronounced GnRH-agonist-associated elevation of LH secretion in the presence of the lower dose of GnRH antagonist would reflect a higher availability of unoccupied pituitary GnRH receptors.

The lower dose of GnRH antagonist (450 μg/kg) was not sufficient to counteract GnRH agonist action significantly in the present study, although the same dose of the Nal-Glu GnRH antagonist alone effectively suppressed bioactive LH and testosterone in this monkey species [25]. Apparently the dose of GnRH antagonist required to prevent the action of GnRH agonist in the non-human primate model is considerably higher (2250 μg/kg daily in the present study) than the dose required for suppression of baseline hormone secretion. In normal men, 5–10 mg Nal-Glu on a daily basis was used to suppress LH and testosterone secretion [9, 14, 15]. In order to achieve a similar effect to that seen in the non-human primate model, relatively high doses of GnRH antagonists, or alterntively GnRH antagonists with increased antigonadotropic potency, will be required for clinical application.

After withdrawal of GnRH antagonist, a second period of GnRH agonist-induced elevation of LH and testosterone secretion was observed. In both groups treated with GnRH antagonist, the rise of LH did not surpass the normal baseline range. However, testosterone levels in the group exposed to the lower dose of GnRH antagonist exhibited levels in the supranormal range 2 days after cessation of administration of GnRH antagonist. Since the present study focused on the initial GnRH agonist-associated hormone stimulation, the frequent (daily) blood sampling was not extended beyond day 15 after initiation of GnRH agonist administration. Therefore, it cannot be excluded from the present data that higher levels of LH and testosterone were present 12 or 24 h after withdrawal of GnRH antagonist, or at a later point. The timing of this second LH rise will probably depend on the disappearance rate of the GnRH antagonist, whereas the magnitude and amount of LH release should depend on the residual pituitary LH concentrations.

Provided that this effect also persists during subsequent monotherapy with GnRH agonist, this might represent another approach to prevent GnRH-agonist-associated flare-up besides the combination therapy with the anti-androgens cyproterone acetate [5] or flutamide [4, 12, 23].

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References

1. Akhtar FB, Wickings EJ, Nieschlag E (1984) Male fertility control with an LH-RH agonist: primate studies. In: Vickery BH, Nestor JJ Jr, Hafez ESE (eds) LH-RH and its analogs. MTP Press, Lancaster, p 77
2. Behre HM, Nashan D, Hubert W, Nieschlag E (1992) Depot gonadotropin-releasing hormone agonist blunts the androgen-induced suppression of spermatogenesis in a clinical trial of male contraception. J Clin Endocrinol Metab 74:84
3. Behre HM, Klein B, Steinmeyer E, Nieschlag E (1991) Effective suppression of luteinizing hormone and testosterone by single doses of the new gonadotropin-releasing hormone antagonist SB-75 in normal men. (Abstract 1155) 73rd Meeting of the Endocrine Society, p 319, Washington, June 18–22, 1991
4. Boccardo F (1990) Total androgen blockade: rationale and review of clinical experience. In: Bouchard P, Haour F, Franchimont P, Schatz P (eds) Recent progress on GnRH and gonadal peptides. Elsevier, Amsterdam, p 293
5. Boccon-Gibod L, Laudat MH, Dugue MA, Steg A (1986) Cyproterone acetate lead-in prevents initial rise of serum testosterone by luteinizing hormone-releasing hormone analogs in the treatment of metastatic carcinoma of the prostate. Eur Urol 12:400
6. Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Dekker, New York
7. Heber D, Dodson R, Swerdloff RS, Channabasavaiah K, Stewart JM (1982) Pituitary receptor site blockade by a gonado-

tropin-releasing hormone antagonist in vivo: mechanism of action. Science 216:420
8. Heber D, Dodson R, Peterson M, Channabasavaiah KC, Stewart JM, Swerdloff RS (1984) Counteractive effects of agonistic and antagonistic gonadotropin-releasing hormone analogs on spermatogenesis: sites of action. Fertil Steril 41:309
9. Jockenhövel F, Bhasin S, Steiner B, River JE, Vale WW, Swerdloff RS (1988) Hormonal effects of a single gonadotropin-releasing hormone antagonist dose in men. J Clin Endocrinol Metab 66:1065
10. Karten MJ, Rivier J (1986) Gonadotropin-releasing hormone analog design. Structure-function studies towards the development of agonists and antagonists: rationale and perspective. Endocr Rev 7:44
11. Loumaye E, Catt KJ (1983) Agonist-induced regulation of pituitary receptors for gonadotropin-releasing hormone. J Biol Chem 258:12002
12. Lunglmayr G (1988) Zoladex vs Zoladex plus flutamide in the treatment of advanced prostate cancer. First interim analysis of an international trial. In: Murphy GP, Khoury S (eds) Therapeutic progress in urological cancers. Liss, New York, p 1451
13. Marshall GR, Akhtar FB, Weinbauer GF, Nieschlag E (1986) Gonadotropin-releasing hormone (GnRH) overcomes GnRH antagonist induced suppression of LH secretion in primates. J Endrōcrinol 110:145
14. Pavlou SN, Wakefield G, Schlechter NL, Lindner J, Souza KH, Kamilaris TC, Kondidaris S, Rivier JE, Vale WW, Toglia M (1989) Mode of suppression of pituitary and gonadal function after acute or prolonged administration of a luteinizing hormone-releasing antagonist in normal men. J Clin Endocrinol Metab 68:446
15. Pavlou SN, DeBold CR, Orth DN (1990) LHRH antagonist: clinical studies in man. In: Bouchard P, Haour F, Franchimont P, Schatz B (eds) Recent progress on GnRH and gonadal peptides. Amsterdam, Elsevier, p 195
16. Puente M, Catt KJ (1986) Inhibition of pituitary-gonadal function in male rats by a potent GnRH antagonist. J Steroid Biochem 25:917
17. Sandow J (1982) Inhibition of pituitary and testicular function by LHRH analogues. In: Jeffcoate SL, Sandler M (eds) Progress towards a male contraceptive. Wiley, Chicester, p 191
18. Sandow J (1983) Clinical applications of LHRH and its analogues. Clin Endocrinol 18:571
19. Santen RJ (1989) Hormonal therapy of prostate cancer: choosing among several available options. Int J Androl 12:165
20. Schmidt F, Sundaram K, Thau RB, Bardin CW (1984) [Ac-D-NAL(2)^1,4FD-Phe^2,D-Trp^3,D-Arg^6]-LHRH, a potent antagonist of LHRH, produces transient edema and behavioral changes in rats. Contraception 29:283
21. Schürmeyer T, Wickings EJ, Freischem CW, Nieschlag E (1983) Saliva and serum testosterone following oral testosterone undecanoate administration in normal and hypogonadal men. Acta Endocrinol 102:456
22. Swerdloff RS, Bhasin S, Salameh W, Tom L, Peterson M, Steiner B (1990) Combined GnRH antagonist and testosterone enanthate for experimental male contraception. (Abstract 35) Gynecol Endocrinol 4:28
23. Waxman J (1989) Short-term and anti-androgen therapy and very long-acting depot gonadotropin-releasing hormone agonist for prostatic cancer. In: Murphy GP, Khoury S (eds) Therapeutic progress in urological cancers. Liss, New York, p 61
24. Weinbauer GF, Nieschlag E (1985) Regulation of primate testicular function by GnRH analogues. Med Biol 63:210
25. Weinbauer GF, Göckeler E, Nieschlag E (1988) Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone antagonist-treated nonhuman primate (*Macaca fascicularis*). J Clin Endocrinol Metab 67:284
26. Weinbauer GF, Jackwerth B, Yoon YD, Behre HM, Yeung CH, Nieschlag E (1990) Pharmacokinetics and pharmacodynamics of testosterone enanthate and dihydrotestosterone enanthate in non-human primates. Acta Endocrinol 122:432
27. Wickings EJ, Qazi MH, Nieschlag E (1979) Determination of biologically active LH in the serum of male rhesus monkeys. J Reprod Fertil 57:497
28. Wynn PC, Suarez-Quian CA, Childs GV, Catt KJ (1986) Pituitary binding and internalization of radioiodinated gonadotropin-releasing hormone agonist and antagonist ligands in vitro and in vivo. Endocrinology 119:1852

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