ELSEVIER

Contraception

Contraception 69 (2004) 505–511
Original research article

The effect of Nestorone on gonadotropic cells in pituitary of rats

Xiao-Feng Zhao[b], Yan-Qun Xu[b], Yan-Ling Wang[a], Ying Leng[b], Lin Cao[b], Jing-De Jin[c], Zhi-Ping Gu[b,*]

[a]*State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China*
[b]*Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China*
[c]*Zhejiang Xianju Pharmaceutical Co., Ltd, Xianju 317300, China*

Received 22 November 2003; received in revised form 20 January 2004; accepted 20 January 2004

Abstract

The implant containing Nestorone is a promising long-acting contraceptive especially suitable for lactating women. In this study, two experiments were designed to observe the effect of Nestorone on the gonadotropic cells in pituitary of rats for analyzing its antiovulation mechanism. In the first experiment, the ED_{50} of Nestorone on inhibiting ovulation was found to be 1.32 mg/kg. The serum luteinizing hormone (LH) levels were significantly lower 60 h after being treated with Nestorone at 8:30–9:00 a.m. on Day 2 (D2) of estrus. Image analysis showed that the average size of the LH cells in groups treated with Nestorone at 2 or 4 mg/kg was larger than that of the control. In the group treated with 4 mg/kg, most of gonadotropic cells were regular round in shape. And, abundant granules in cytoplasm were found in those cells, which indicated that the LH stored in cells was not released. In the second experiment, the rats were treated with Nestorone at 5 mg/kg at 11:30–12:00 a.m. on D2 of estrus. The normal or higher expression of LHβ mRNA in pituitary suggested that the synthesis of LH was not inhibited by the treatment with Nestorone. The expression of PR mRNA in pituitary was significantly lower than that of the control at 33 h after treatment. This might be a direct effect of Nestorone, since there were no differences in the serum E_2 and P_4 levels between the treated and the control group. It is concluded that Nestorone prevents ovulation through inhibition of LH secretion and it has no effect on synthesis of LH. Progesterone receptors in pituitary might be involved in this process, but further study is needed to gain more evidence.

Keywords: Nestorone; LH; Gonadotropic cells; LHβ mRNA; PR mRNA

1. Introduction

Nestorone (16-methylene-17α-acetoxy-19-nor-4-pregnene-3,20-dione), formally called ST-1435, is a potent progestin administered parenterally. Upon oral administration, Nestorone undergoes rapid metabolism and inactivation because of rapid hepatic first-pass effect. When given parenterally via sustained release formulations such as subdermal implant, vaginal ring and transdermal administration, etc., a strong progestational activity combined with lack of androgenic, estrogenic and glucocorticoid-like activities confer special advantages to this steroid for use in contraception. Since the 1970s, a subdermal implant releasing this progestin has been in development for female contraception, which is convenient to use, long-lasting, highly efficacious and safe. In the clinical trials, Nestorone was found to be very effective in controlling fertility at a low dose. In addition, this implant is recommended as especially suitable for contraception during lactation. The fetal liver is capable of metabolizing Nestorone and the hepatic first-pass effect is so strong that the steroid transferred through breast milk should be metabolized as inactive products that have no progestational effect and should pose no risk to the infant [1–7].

In clinical trails, it has been demonstrated that the contraceptive efficacy of Nestorone is mainly due to its inhibitory effect on ovulation [8,9]. In the manner of concentration-dependence, lower plasma concentrations of Nestorone act on the hypothalamus and/or pituitary, whereas at higher plasma concentrations, a direct effect on the ovaries is also achieved [9]. Our earlier study showed that Nestorone could not inhibit the superovulation induced by pregnant mare

* Corresponding author. Tel.: +86-21-5080-6600, ext. 2515; fax: +86-21-5080-7088.
E-mail address: zpgu99@yahoo.com (Z.-P. Gu).

0010-7824/04/$ – see front matter
doi:10.1016/j.contraception.2004.01.007

serum gonadotropin and human chorionic gonadotropin, while it prevented the ovulation induced by gonadotropin-releasing hormone (GnRH) in mature rats [10]. This suggested that the pituitary is the main target organ upon which Nestorone acts. In this study, the first experiment was designed to observe the effects of Nestorone on the function and morphology of pituitary gonadotropic cells in rats.

We learned from the literature that the progesterone receptor (PR) and the GnRH receptor (GnRH-R) were involved in regulation of preovulatory gonadotropin secretion in the anterior pituitary of female rats [11,12]. Colocalization of the PR protein with the β-subunit of luteinizing hormone (LH) in the gonadotropic cells suggested a key role of PR in direct regulation of gonadotrophin secretion. In addition, the hypothalamic peptide GnRH is the primary neuroendocrine signal regulating cyclic gonadotropin secretion in the female. GnRH regulates LH synthesis and secretion through the high affinity GnRH-R on the plasma membranes of pituitary gonadotropic cells. The GnRH-R number is correlated with the magnitude of gonadotropin secretion responses to GnRH. The increase in GnRH-R levels before preovulation likely plays an important role in enhancing pituitary sensitivity to GnRH. The second experiment was designed to determine the effects of Nestorone on mRNA expression of PR B, PR A+B (total PR), GnRH-R and LHβ in pituitary of rats.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats were housed in an air-conditioned room under 14-L:10-D schedule, with lights on at 0600 h. They were provided with standard rat chow and tap water ad libitum. Vaginal smears were performed every morning and only the rats showing at least two consecutive regular 4-day estrous cycles were used in this study.

2.2. Compounds and reagents

Nestorone was manufactured by Zhejiang Xian ju Pharmaceutical Co., Guinea pig anti-rat LHβ antibody and radioimmunoassay (RIA) kits for LH measurement in rats were supplied by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, Bethesda, MD, USA). Biotinlyated goat anti-guinea pig IgG and ABC kits were purchased from Vector Laboratories (Burlingame, CA, USA). 3′,3-Diaminobenzidine (DAB) staining kits were from Sino-American Biotechnology, TRIzol reagent was from Gibco (Grand Island, NY, USA), random hexamers, AMV reverse transcriptase and *Taq* DNA polymerase were from Promega (Madison, WI, USA). Rat estradiol (E_2) and progesterone (P_4) RIA kits were obtained from Beckman Coulter (Fullerton, CA, USA).

2.3. Antiovulation test

Rats were given a single subcutaneous injection with various doses of Nestorone or megestrol acetate at 11:30–12:00 a.m. on Day 2 (D2) of estrus (n = 10/group). Peanut oil was administrated to rats in the control group. Rats were sacrificed at 72 h after administration, and the oviducts were separated. Ova were rinsed out and counted under the dissecting microscope. The median effective dose (ED_{50}) of Nestorone on inhibiting ovulation in rat was calculated by the Bliss method.

2.4. Analysis of the function and morphology of rat pituitary gonadotropic cells

Rats were treated by subcutaneous injection with 1.0, 2.0 or 4.0 mg/kg of Nestorone or peanut oil at 8:30–9:00 a.m. on D2 of estrus (n = 9–10/group). Sixty hours after administration (late proestrus in the control), rats were sacrificed and blood samples were collected for measurement of serum LH. The pituitaries were enucleated and fixed in Bouin's solution. Three pituitaries from each group were selected randomly for dehydrating and embedding in paraffin. Coronal sections (5 μm) were made and mounted onto glass slides for immunohistochemical staining.

2.5. Immunohistochemical analysis

Immunohistochemical staining of pituitary gonadotropic cells containing LH was performed according to the method described by Zhou et al. [13]. Briefly, sections were deparaffinized, hydrated and treated with 3% hydrogen peroxide to block the endogenous peroxidase activity. Nonspecific background staining was suppressed by incubation with 0.3% bovine serum albumin and normal goat serum. Then the sections were incubated with guinea pig anti-rat LHβ antibody at a dilution of 1:6000 for 45 min at 37°C in a humidified chamber. After washing in 0.1 mol/L phosphate-buffered saline, the sections were incubated with biotinylated goat anti-guinea pig IgG (1:200, 37°C, 20 min) followed by avidin-biotin-peroxidase complex (1:100, 37°C, 20 min) from a Vector ABC kit. Finally, the pituitary gonadotropic cells that contained LH were visualized by incubating with a DAB of 0.5 g/L for 10 min at 25°C. A universal microspectrophotometer combined with a KS400 Image Analysis System (Zeiss, Germany) was used to analyze the cell size and shape.

2.6. Analysis of PR A+B, PR B, GnRH-R and LHβ mRNA levels in pituitary

Rats were treated by intramuscular injection with Nestorone of 5 mg/kg (ED_{90} of antiovulation) at 11:30–12:00 a.m. on D2 of estrus. Peanut oil was administered to rats of the control group. Animals were sacrificed by decapitation at 33 h, 45 h, 57 h and 69 h after treatment (n = 5/time point). Pituitary was rapidly removed, frozen in liquid nitrogen and

Table 1
Sequences of primers and cycles used in RT-PCR analysis of PR A + B, PR B, GnRH-R and LHβ mRNA

Gene	Primer sequence (5′→3′)		Positions (nt)	Product size (bp)	PCR cycles
PR A+B	Sense:	CCCACAGGAGTTTGTCAAGCTC	3009–3030	326	25
	Antisense:	TAACTTCAGACATCATTTCCGG	3313–3334		
PR B	Sense:	GTGTGAGGATTCTGCCTTTC	40–59	221	27
	Antisense:	CGCTCTCAGGACTTCTTACG	241–260		
GnRH-R	Sense:	GGAGAAATATGGCTAACAATGC	719–740	271	25
	Antisense:	AGGTTGGCTAAGGTCAAATG	970–989		
LHβ	Sense:	CTTCACCACCAGCATCTGTG	136–155	250	25
	Antisense:	GTCACAGGTCATTGGTTGAG	366–385		
GAPDH	Sense:	ACCACAGTCCATGCCATCAC	1369–1388	452	23
	Antisense:	TCCACCACCCTGTTGCTGTA	1801–1820		

stored at −80°C until RNA extraction. Blood samples were collected for RIA of serum hormone levels.

2.7. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from whole anterior pituitary using TRIzol reagent according to the manufacturer's instructions. Approximately 4 μg of total RNA was reverse transcripted into cDNA with 30U AMV reverse transcriptase and random hexamers (0.5 g/g total RNA) to prime the reaction at 37°C for 60 min.

The primers used for PCR amplification are summarized in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used in the same sample as an internal control. PCR amplifications were performed in two steps according to the previous description [11]. In the first step, the reaction mixture contained RT reaction product 2.5 μL, each primer 0.4 μmol/L, and 1U *Taq* DNA polymerase in a final volume of 12.5 μL. After the appropriate number of cycles with the first set of primers, 12.5 μL of another mixture containing the primers for GAPDH 0.4 μmol/L, and an additional 1U *Taq* DNA polymerase was added for the remaining cycles. At the end of the last cycle, an additional extension at 72°C for 10 min was conducted. The PCR products were separated by electrophoresis on 2% agarose gel containing ethidium bromide (Fig. 1). Imaging Digital System (United-Bio, Marlton, NJ, USA) was used to quantify the signal intensity, and the values were expressed as ratios of each gene to GAPDH.

2.8. Hormone assay

LH concentration in the serum was measured by means of RIA using the rat LH kits kindly supplied by NIDDK. Serum E_2 and P_4 were measured by RIA kits purchased from Beckman Coulter.

2.9. Statistical analysis

All data were expressed as mean ± SD. The significance of difference between treated group and control group was determined by the Student's t test.

3. Results

3.1. ED_{50} of antiovulation

Both Nestorone and megestrol acetate suppressed the ovulation of rats effectively when administrated subcutaneously on D2 of estrus. The ED_{50} of Nestorone is 1.32 mg/kg, while the ED_{50} of megestrol acetate is 2.17 mg/kg. Comparing by the ED_{50}, the antiovulation potential of Nestorone is 1.6 times that of mesgetrol acetate (Table 2).

3.2. Effect on the function and morphology of gonadotropic cells

3.2.1. Serum LH level

Serum LH concentration in control rats was 3.33 ± 2.57 ng/mL. In the groups treated with Nestorone at 1.0, 2.0 or 4.0 mg/kg, the serum LH levels were 0.99 ± 1.23, 0.29 ±

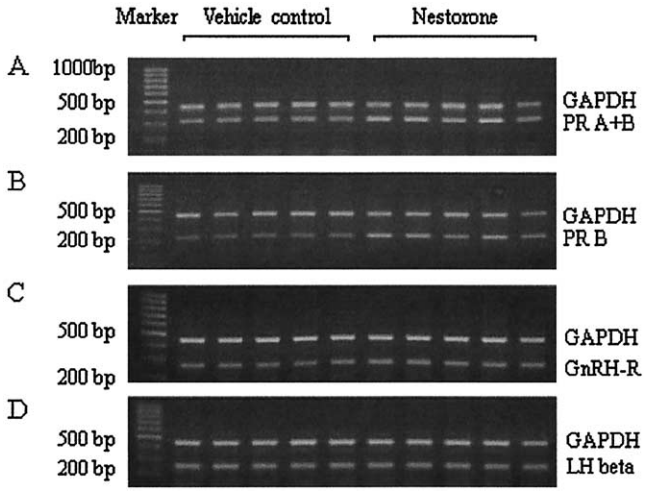

Fig. 1. RT-PCR analysis of PR A+B, PR B, GnRH-R and LHβ mRNA expression in the pituitary of rats. PCR products were run and separated by 2% agarose gel. (A) 326-bp PCR product of PR A+B; (B) 221-bp product of PR B; (C) 271-bp product of GnRH-R; (D) 250-bp product of LHβ. Reverse-transcribed RNA from pituitaries of rats collected at 69 h after treatment. The internal control, GAPDH, amplified for 23 cycles in every reaction, yielded a 452-bp product.

Table 2
Effect of Nestorone on ovulation of rats

Group	Dose (mg/kg)	No. of tested rats	No. of ovulatory rats	Inhibitory rate of ovulation (%)	ED_{50} (95% confidence interval) mg/kg
Control	0	10	10	0	
	0.5	10	9	10	
Nestorone	1	10	5	50	1.32 (0.49–3.51)
	2	10	3	70	
	4	10	2	80	
Megestrol acetate	1	10	8	20	
	2	10	6	40	2.17 (0.95–4.96)
	4	10	2	80	

0.11 and 0.33 ± 0.16, respectively, which were significantly lower than that of the control (Table 3).

3.2.2. Size and shape of gonadotropic cells in pituitary

After immunohistochemical staining, the pituitary gonadotropic cells containing LH could be identified by the appearance of brown granules. In the control group, the gonadotropic cells were small and irregular in shape. No obvious differences of the size and shape of gonadotropic cells were found between the rats treated with Nestorone 1.0 mg/kg and the control, while larger and regular cells were observed in the rats treated with Nestorone 2.0 mg/kg. In the group of 4.0 mg/kg, most of the gonadotropic cells were large and round, particularly, a huge amount of brown granules were found in the cytoplasm of cells, which suggested that large quantities of LH were stored in these cells (Fig. 2).

The results of image analysis showed that the average areas of pituitary gonadotropic cells in the groups treated with Nestotone at 2.0 or 4.0 mg/kg were significantly larger than that of the control group ($p < 0.001$). In control rats, most of the gonadotropic cells were small, and the areas of 42.2% of cells were less than 60 μm^2. In treated groups, the amount of small cells decreased and large cells increased with the increased dosage of Nestorone. When the dosage of Nestorone increased to 4.0 mg/kg, only 13.8% of gonadotropic cells had an area of less than 60 μm^2, while the area of 48.7% of the cells were 80~120 μm^2. This was 2.14 times the area of the control (Table 4).

Another parameter, named circle factor, describes the degree of deviation from a circle of the cross-section of cells. When the cross-section of a cell is a circle, the circle factor is equal to 1; for cross section of a cell is a circle, the circle factor is equal to 1, for cross section of a cell not a circle, the circle factor is less than 1. The more irregular the cell, the smaller the circle factor. Results of the image analysis showed that the average circle factors in Nestorone-treated groups were higher than that of the control, though a significant difference was only observed between the group of rats treated with Nestorone 4.0 mg/kg and control ($p < 0.01$). The circle factors of most gonadotropic cells in each group were 0.6–0.8. In the control group, the circle factor of 19.5% of cells was less than 0.6, while only 10.5% of cells that had a circle factor less than 0.6 existed in the group treated with Nestorone 4.0 mg/kg. In addition, the circle factor of 12.3% of cells in the control group was higher than 0.8, while 18.5% of cells in the rats treated with Nestorone 4.0 mg/kg had that large circle factor (Table 5).

Table 3
Serum LH concentration at 60 h after treatment with Nestorone in rats (n = 9)

Group	Dose (mg/kg)	LH concentration (ng/mL)[a]
Control	0	3.33 ± 2.57
	1	0.99 ± 1.23*
Nestorone	2	0.25 ± 0.11**
	4	0.33 ± 0.16**

[a] Values are mean ± SD.
* $p < 0.05$ vs. control.
** $p < 0.01$ vs. control.

3.3. Expression of PR A+B, PR B, GnRH-R and LHβ mRNA in pituitary

In rats treated with Nestorone 5.0 mg/kg at 1130–1200 h on D2 of estrus, the expression of PR A+B mRNA and PR B mRNA in the pituitary was normal at 45 h after treatment, but obviously lower at 33 h and markedly higher at 57 and 69 h when compared to the control (Figs. 3A and B).

The expression of GnRH-R mRNA in the pituitary was significantly higher in the Nestorone-treated group than in the control group at 45 and 69 h, and there was no significant difference from the control at 33 h and 57 h after treatment (Fig. 3C).

Compared to the control, a higher expression of LHβ mRNA in the pituitary of the treated group was observed at 57 h after treatment. No difference was found between the control and treated group at 33 h, 45 h and 69 h after treatment (Fig. 3D).

3.4. Serum E_2 and P_4 levels

Compared with the control rats, the serum E_2 levels in the treated group were normal at 33 h and observably lower

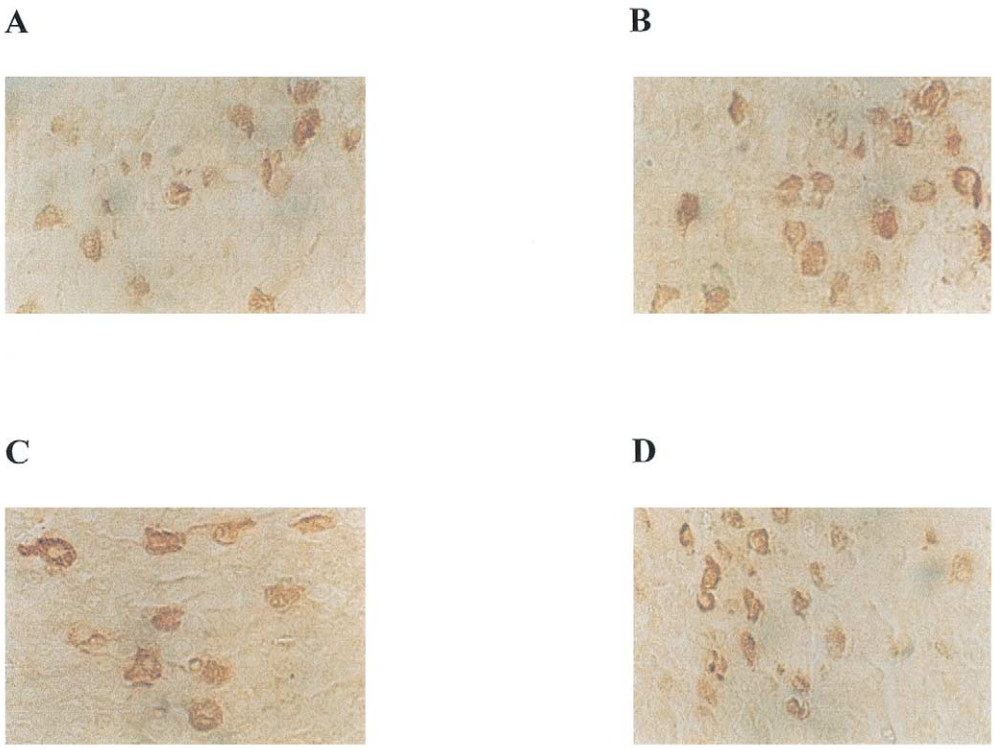

Fig. 2. Effects of Nestorone on the morphology of gonadotropic cells in pituitary of rats. Animals were treated subcutaneously with various dose of Nestorone or vehicle at 8:30–9:00 a.m. on D2 of estrus. At 60 h after treatment, rats were decapitated and paraffin sections of pituitaries were stained by ABC technique of immunohistochemistry. Brown granules in gonadotropic cells were observed under the microscope (×500): (A) 1.0 mg/kg; (B) 2.0 mg/kg; (C) 4.0 mg/kg; (D) vehicle control.

($p < 0.01$) at 45 h after administration, while higher levels of serum E_2 were found in the treated group ($p < 0.05$) at 57 and 69 h. The serum concentrations of P_4 in the treated groups were lower than in the control during the period of observation, especially at 57 h after treatment ($p < 0.01$) (Table 6).

4. Discussion

As the intermediate of hypothalamus-pituitary-ovary axis, the pituitary plays a key role in the regulation of reproductive function in mammals. The LH peak in mid menstrual cycle is a main initiating factor of ovulation. In this study, two experiments were designed to observe the functional and morphological changes of gonadotropic cells, as well as the expression of PR mRNA, GnRH-R mRNA and LHβ mRNA in pituitary of the rats after treatment with Nestorone. The results of the first experiment showed that the serum LH levels in treated groups were significantly lower than that of the control at 60 h after treatment, indicating suppression of the preovulation LH release.

In female adult rats, the gonadotropic cells of the pituitary secrete two kinds of gonadotropins, namely LH and follicle-stimulating hormone (FSH). There are about 60% of gonadotropic cells secreting both of LH and FSH, while 18% and 23% of gonadotropic cells only secrete LH or

Table 4
The size of gonadotropic cells in pituitary at 60 h after treatment with Nestorone in rats

Dose of Nestorone (mg/kg)	No. of rats	No. of sections	No. of cells	Cell area (μm^2)[a]	Distribution of cell area (%)					
					<60	<80	<100	<120	<150	≥150
0	3	15	154	68.116 ± 32.620	42.2	25.3	15.2	6.0	8.4	1.3
1	3	15	168	72.091 ± 30.139	38.7	25.6	18.4	10.7	5.4	1.2
2	3	15	160	84.982 ± 33.647***	25.0	20.0	24.4	15.6	12.5	2.5
4	3	15	152	91.935 ± 30.027***	13.8	23.0	25.7	23.0	10.5	4.0

[a] Values are mean ± SD.
***$p < 0.001$ vs. control.

Table 5
Circle factors of gonadotropic cells in pituitary at 60 h after treatment with Nestorone in rats

Dose of Nestorone (mg/kg)	No. of rats	No. of sections	No. of cells	Circle factor[a]	Distribution of circle factor of cells (%)					
					<0.5	<0.6	<0.7	<0.8	<0.9	≥0.9
0	3	15	154	0.683 ± 0.107	5.2	14.3	30.5	37.7	11.7	0.6
1	3	15	168	0.687 ± 0.110	5.4	14.3	31.0	33.9	14.9	0.6
2	3	15	160	0.703 ± 0.102	3.1	11.9	30.0	36.9	15.0	0.3
4	3	15	152	0.717 ± 0.091**	1.3	9.2	26.3	44.7	17.8	0.7

[a] Values are mean ± SD.
**$p < 0.01$ vs. control.

FSH, respectively [14]. During the estrous cycle, the morphology of gonadotropic cells in the pituitary varies. The morphological changes of these cells could reflect the synthetic and secretory state of LH in the pituitary [13,15]. In the present study, the pituitaries were collected at late proestrus of rats in which the preovulation LH surge had been released. In the control rats, the small size and irregular shape of the gonadotropic cells in the pituitary suggested that the LH stored in these cells had been released, which was confirmed by the high LH level in the serum. However, the gonadotropic cells in the pituitary of treated groups were large in size and were more regular in shape. Furthermore, in the group treated with Nestorone 4.0 mg/kg, abundant granules were found in the cytoplasm of the gonadotropic cells. This indicated that LH had not been released from these cells, which was coincident with the low level of serum LH in treated rats. Therefore, it is suggested that the antiovulation effect of Nestorone is mainly due to its effect on the inhibition of LH release.

In the second experiment, the serum E_2 and P_4 peak found in the control rats at 45 h and 57 h, respectively, after treatment suggested the occurrence of ovulation. On the contrary, no P_4

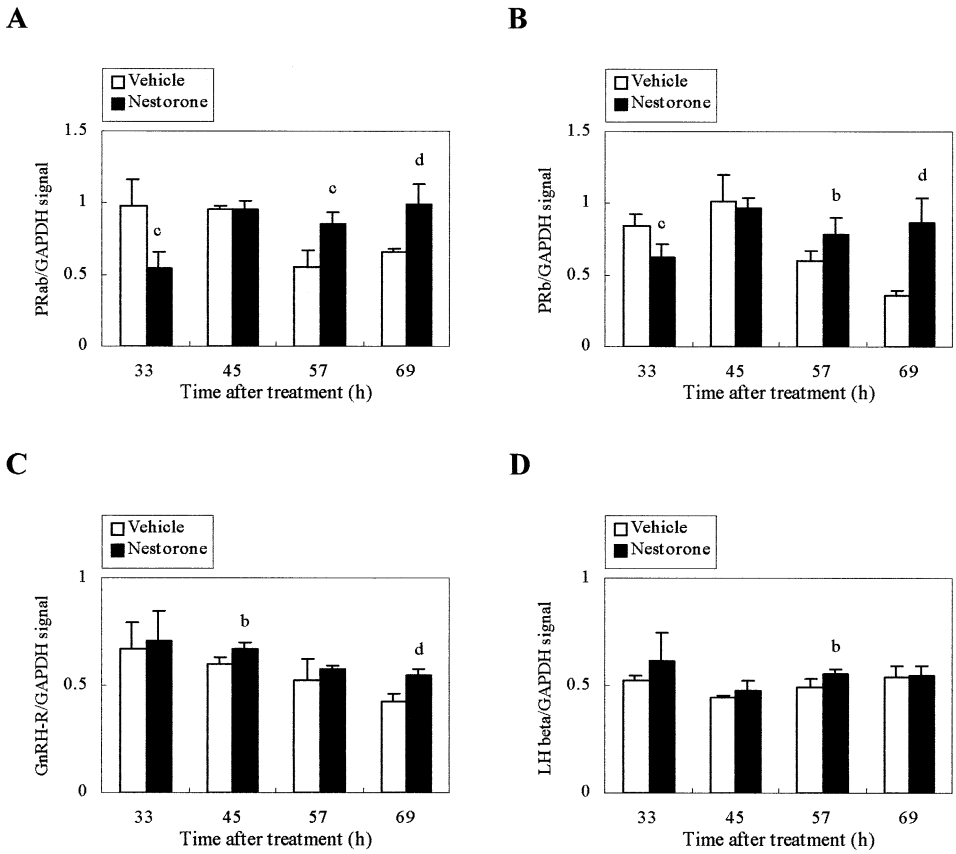

Fig. 3. Effects of Nestorone on mRNA expression of PR, GnRH-R and LHβ in pituitary. Rats were treated with intramuscular Nestorone 5 mg/kg or vehicle at 11:30–12:00 a.m. on D2 of estrus. Total RNA for RT-PCR were isolated from pituitary at 33 h, 45 h, 57 h and 69 h after treatment. Data are represented as mean ± SD (n = 5) of PR A+B/GAPDH (A), PR B/GAPDH (B), GnRH-R/GAPDH (C) and LHβ/GAPDH (D). $^{b}p < 0.05$; $^{c}p < 0.01$; and $^{d}p < 0.001$ vs. controls at the same time points.

Table 6
Concentrations of serum E_2 and P_4 at 60 h after treatment with Nestorone in rats (n = 5)

Dose of Nestorone (mg/kg)	E_2 (pg/mL)[a]				P_4 (ng/mL)[a]			
	Time after treatment (h)				Time after treatment (h)			
	33	45	57	69	33	45	57	69
0	8.8 ± 3.493	37 ± 11.958	3.2 ± 2.950	2.4 ± 3.782	26.464 ± 2.541	23.322 ± 9.120	>40	21.438 ± 4.225
5	4.6 ± 5.459	12 ± 6.753**	35 ± 24.658*	39 ± 28.381*	25.998 ± 8.137	18.644 ± 6.826	27.444 ± 5.934**	17.45 ± 4.029

[a] Values are mean ± SD.
* $p < 0.05$ vs. controls.
** $p < 0.01$ vs. controls.

peak in serum was found in the treated group. This indicated that ovulation was prevented in the cycle following treatment with Nestorone. However, the higher or normal expression of LHβ mRNA in pituitary suggested that the synthesis of LH was not suppressed by the treatment of Nestorone. This is coincident with the result of the abundant granules found in the cytoplasm of gonadotropic cells.

In the pituitary, PR and GnRH-R act as important factors involved in the regulation of preovulation LH surge. PR may regulate LH secretion directly, and P_4 enhances the response of pituitary to GnRH mediated by PR. In cultured anterior pituitary cells of ovariectomized rats, PR mRNA was up-regulated by E_2 and acutely down-regulated by P_4. In addition, E_2 increases GnRH-R and the sensitivity of the pituitary to GnRH [12,16]. Therefore, the mRNA expression of PR and GnRH-R in the pituitary as well as serum levels of E_2 and P_4 were observed in this study. At 33 h after treatment, the mRNA expression of PR was significantly lower than that of the control. This might be a direct effect of Nestorone, since there were no differences in the levels of serum E_2 and P_4 between the treated and the control group. The serum E_2 level of the treated group was observably lower than that of the control at 45 h after administration, indicating a suppression of follicle development. Nestorone may suppress follicle development directly or through suppressing the synthesis and/or secretion of FSH in the pituitary. The E_2 level elevated gradually and even reached a high level over that of the control, indicating renewed follicle development because of the withdrawal of medication. The increasing mRNA expression of PR B, PR A+B, GnRH-R and LHβ coincided with the elevating E_2 level, suggesting the next ovulation cycle was beginning.

In summary, the antiovulation mechanism of Nestorone is mainly due to its inhibiting effect on LH secretion; PR might be involved in this process. In addition, there is no effect of Nestorone on synthesis of LH in the pituitary.

Acknowledgments

We thank Prof. Zhuang Lin-Zhi, State Key Laboratory of Reproductive Biology, and Prof. Zhou Shou-Kang, Shanghai Institute of Planned Parenthood Research, for their significant comments on this study.

References

[1] Lahteenmaki PL. Intestinal absorption of ST-1435 in rat. Contraception 1984;30:143–51.
[2] Noe G, Salvatierra A, Heikinheimo O, Maturana X, Croxatto HB. Pharmacokinetics and bioavailability of ST 1435 administered by different routes. Contraception 1993;48:548–56.
[3] Heikinheimo O, Noe G, Haukkamaa M, Lahteenmaki P. The progestin ST 1435—rapid metabolism in man. Contraception 1994;50:275–89.
[4] Coutinho EM, Athayde C, Dantas C, Hirsch C, Barbosa I. Use of a single implant of elcometrine (ST-1435), a nonorally active progestin, as a long-acting contraceptive for postpartum nursing women. Contraception 1999;59:115–22.
[5] Heikinheimo O, Ranta S, Moo-Young A, Lahteenmaki P, Gordon K. Parenteral administration of progestin Nestorone to lactating cynomolgus monkeys: an ideal hormonal contraceptive at lactation? Hum Reprod 1999;14:1993–7.
[6] Kumar N, Koide SS, Tsong Y, Sundaram K. Nestorone: a progestin with a unique pharmacological profile. Steroids 2000;65:629–36.
[7] Sivin I, Moo-Young A. Recent developments in contraceptive implants at the Population Council. Contraception 2002;65:113–9.
[8] Laurikka-Routti M, Haukkamaa M. A contraceptive subdermal implant releasing the progestin ST-1435: ovarian function, bleeding patterns, and side effects. Fertil Steril 1992;58:1142–7.
[9] Lahteenmaki PL, Lahteenmaki P. Concentration-dependent mechanisms of ovulation inhibition by the progestin ST-1435. Fertil Steril 1985;44:20–4.
[10] Chu ZW, Wang WC, Wang YE, You GD, Gu ZP. Antifertility effect of the implant containing 16-methylene-17-alpha-acetoxy-19-norprogesterone (ST- 1435). Acta Pharmacol Sin 1991;12:285–8.
[11] Szabo M, Kilen SM, Nho SJ, Schwartz NB. Progesterone receptor A and B messenger ribonucleic acid levels in the anterior pituitary of rats are regulated by estrogen. Biol Reprod 2000;62:95–102.
[12] Bauer-Dantoin AC, Weiss J, Jameson JL. Roles of estrogen, progesterone, and gonadotropin-releasing hormone (GnRH) in the control of pituitary GnRH receptor gene expression at the time of the preovulatory gonadotropin surges. Endocrinology 1995;136:1014–9.
[13] Zhou SK, Mao QF, Zhao W, et al. An image quantitative analysis of the morphological changes of LH cells in the female rats during the estrous cycle. Acta Anatomica Sin 1996;27:423–8.
[14] Gu ZP, Sang GW, Chen JK. Reproductive pharmacology. In: Wang MH, editor. Gonadotropin. Anhui (China): Education Publication, 1990. p. 11–8.
[15] Zhou SK, Zhao W, Mao QF, et al. An image analysis of the morphological changes of LH cells during LH release peak induced by LHRH in male rats. Acta Physiologica Sin 1997;49:88–94.
[16] Mahesh VB, Brann DW. Regulation of the preovulatory gonadotropin surge by endogenous steroids. Steroids 1998;63:616–29.