The Effect of Various Dosages of Lynestrenol on the Plasma Levels of Oestrogens and Progesterone during the Menstrual Cycle in the Rhesus Monkey.

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ABSTRACT

The effect of daily oral doses of lynestrenol on the ovarian function was investigated in nineteen rhesus monkeys. The compound was given in four dose levels 0.05, 0.25, 1 and 2.5 mg daily throughout the menstrual cycle. Plasma samples collected before, during and after treatment were analysed for their content of estrogen and progesterone. The low doses 0.05 and 0.25 had no effect on the occurrence of ovulation, the plasma levels of the ovarian steroids being similar to those seen during normal ovulatory cycles. Lynestrenol treatment at doses of 1 and 2.5 mg per day prevented ovulation. The estrogen levels in monkeys treated with 1 mg lynestrenol appeared normal, but the progesterone levels were never more than 0.4 ng/ml. In the monkeys treated with 2.5 mg lynestrenol the estrogen levels were below the lowest levels of the control cycles and the progesterone levels were less than 0.2 ng/ml. The female rhesus monkey appears to require about ten times more lynestrenol per kg body weight than women for suppression of ovulation. However, the plasma levels of norethindrone, which is the active metabolite of lynestrenol, required for ovulation inhibition does not appear to be grossly different from those found in women.

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The rhesus monkey is an experimental animal with promise for use as a test animal in the development of contraceptive agents for human use. The regulation of the reproductive system is fairly well understood in the female rhesus monkey (1). In order to evaluate the usability of the rhesus monkey in the development of contraceptive agents we thought it useful to study wellknown substances with antifertility effect in women through ovulation suppression. Lynestrenol* was synthesized in 1959 and is currently used as a steroidal contraceptive both in combination with estrogens or alone (2, 3). In this paper we report on the effect of four oral dose levels of lynestrenol given daily on the plasma levels of the ovarian steroids and the occurrence of ovulation in the cyclic rhesus monkey. A rapid radioimmunoassay method using antisera prepared in sheep against 11α-hydroxyprogesterone was used for monitoring the plasma progesterone concentrations.

MATERIAL and METHODS

Nineteen mature adult female rhesus monkeys with regular menstrual cycles were used. In the initial trials, two groups of five monkeys each were treated with 0.05 and 0.25 mg lynestrenol daily throughout the menstrual cycle. In the second trial another two groups consisting of four and five monkeys were treated with daily oral doses of 1 and 2.5 mg of lynestrenol during the cycle. The schedule of administration of lynestrenol to the monkeys is summarized in the Table. The lynestrenol tablets were supplied by Organon Oss. The purity specifications were the same as for tablets used in humans. Daily blood samples were collected during the control cycles. The compound was administered in a banana between 8 and 10 a.m. daily during the treatment cycles. Blood samples were collected every other day during the treatment cycles and during the recovery cycles. The estrogen levels in the samples were measured by a rapid radioimmunoassay method (4). No cross reaction was found from norethindrone or lynestrenol. Norethindrone was also measured by radioimmunoassay (5). Unfortunately the study was planned and carried through before this assay was visualized. Therefore it could mainly be used to make sure that the monkeys took their pills.

The behaviour of an antiserum to 11α-hydroxyprogesterone hemisuccinate-BSA raised in sheep, in a rapid radioimmunoassay method described for progesterone (6) was tested and used for measuring the levels of progesterone in the samples. The preparation of 11α-hydroxyprogesterone-hemisuccinate and the BSA conjugate was done in a similar manner as published by Erlanger *et al.* (7). Two sheep were given intramuscular injections of 3 mg 11α-hydroxyprogesterone-succinyl-BSA in 3 ml of saline and suspended in an equal volume of Freund's complete adjuvant once a week for four weeks, followed by monthly booster doses during six months. Blood samples (100 ml) were drawn one week after each monthly booster dose and tested for titre of the antiserum. After the sixth monthly booster dose, when it was observed that the titre of the antiserum did not show any increase over the previous sample, 500 ml of blood was drawn and the sheep were slaughtered. Both sheep had shown signs of severe illness after the third monthly booster dose, and at postmortem the carcasses contained widespread areas of microabscesses. One batch of antiserum from one sheep (Ab FO 22.5) was diluted to 1:1,500 and used for the assay. The dilution gave a usable standard curve between 50 pg and 5 ng. The assay procedure was essentially similar to that described by Thorneycroft and Stone (6) with slight modifications (8). Twentyfive to 200μl of plasma depending on the expected concentration of progesterone were extracted with 10 volumes of petroleum ether. The extraction volume was never less than 1 ml. Dextran coated charcoal 0.5 ml was used to separate the free from the bound steroids. The crossreaction was similar to that of Thorneycroft and Stone (6) with the exception of low, < 1 %, crossreaction of 11-des-oxycortisol. The practical detection limit of the method was 50 pg as read on the standard curve. The plasma blank was less than 50 pg.

* Lynestrenol: 17α-ethinyl-oestr-4-en-17β-o1.

RESULTS

The number of animals, doses, the schedule of administration and the menstrual cycle characteristics are summarized in the Table.

According to the plasma levels of progesterone,ovulation occurred during the treatment cycles in all the monkeys receiving 0.05 and 0.25 mg lynestrenol per day. The treatment cycle lengths were identical in four of the five monkeys treated with 0.05 mg per day. In the monkeys receiving 0.25 mg lynestrenol per day the treatment cycle lengths were identical to (2), longer (2) or shorter (1) than the control cycle lengths.

Figures 1 and 2 illustrate the patterns of the steroids in two monkeys before, during and after treatment with 0.05 and 0.25 mg lynestrenol daily throughout the menstrual cycle. During treatment with 0.05 mg lynestrenol (Fig. 1) very little changes occurred in the steroid levels. Both estrogens and the progesterone levels were suggestive of ovulations and the formation of a normal corpus luteum before, during and after

TABLE. Schedule of lynestrenol administration and menstrual cycle characteristics of the rhesus monkeys used. SA indicates the onset of summer amenorrhoea.

Monkey No.	Control cycles		Treatment cycles				Recovery cycles	
	Length Days	Status	Doses	Duration of treatment Days of cycle	Length Days	Status	Length Days	Status
79	33	Ovul.	0.05 mg	1 – 30	31	Ovul.	26	Ovul.
99	31	"	per day	1 – 31	31	"	32	"
80	25	"		1 – 24	25	"	30	"
118	26	"		1 – 25	26	"	24	"
96	29	"		1 – 28	29	"	SA	SA
102	25	"	0.25 mg	1 – 24	25	"	25	Ovul.
122	26	"	per day	1 – 25	26	"	25	"
94	28	"		1 – 28	32	"	33	Not determined
97	25	"		1 – 21	22	"	22	Ovul.
125	26	"		1 – 26	29	"	31	Not determined
96	28	"	1 mg/	1 – 27	31	Anov.	30	Ovul.
119	28	"	day	1 – 28	31	"	29	Not determined
121	29	"		1 – 18	19	"	29	Ovul.
123	31	"		1 – 31	34	"	25	Not determined
90	30	"	2.5 mg	7 – 30	30	"	27	Ovul.
114	28	"	per day	7 – 28	34	"	25	"
121	27	"		5 – 27	32	"	26	"
97	25	"		6 – 25	SA	SA	SA	Anov.
125	26	"		4 – 26	35	Anov.	SA	"

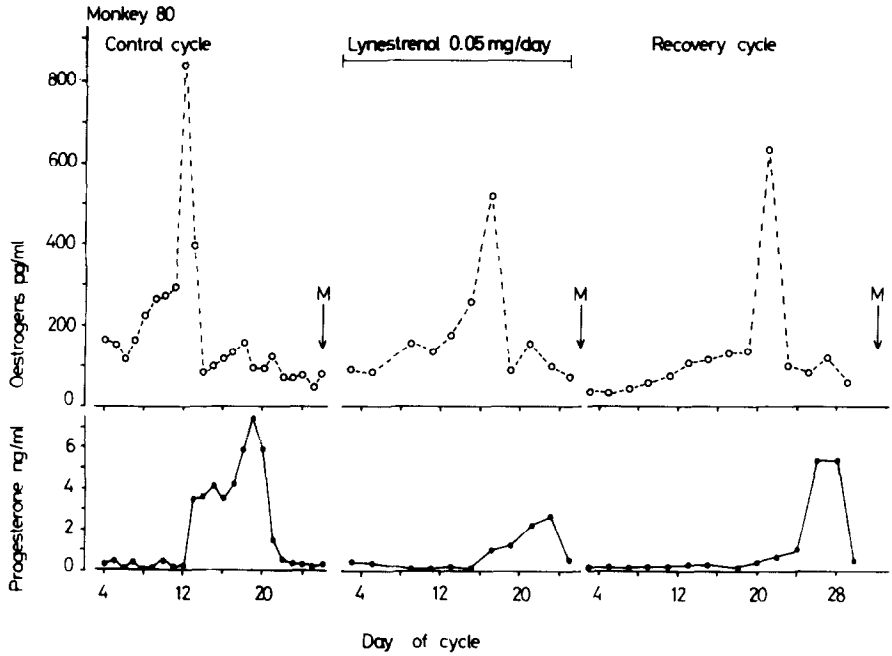

Fig. 1. Peripheral plasma levels of progesterone and oestrogens before, during and after lynestrenol treatment (0.05 mg/day) in a rhesus monkey. M indicates the onset of menstruation.

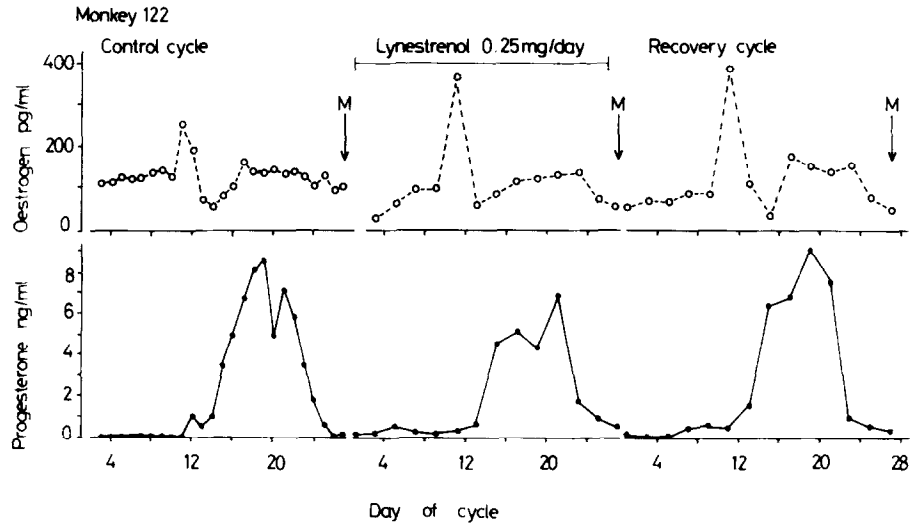

Fig. 2. As in Fig. 1 during treatment with 0.25 mg of lynestrenol daily.

treatment. The progesterone levels during the treatment cycle were, however, slightly lower than the levels recorded during the control and recovery cycles.

During treatment with 0.25 mg lynestrenol, the results of the measurement of the plasma levels of estrogens and progesterone were indicative that both ovulation and development of a normally active corpus luteum had occurred in all the monkeys (e.g. Fig. 2). The typical midcycle estrogen peaks and the luteal progesterone complex suggestive of ovulation were seen in all the monkeys.

Treatment with 1 mg lynestrenol resulted in anovulation in all four monkeys. The treatment cycle lengths were three days longer in three of the monkeys, whilst in the fourth monkey the treatment cycle length was 10 days less than the control cycle length. The cycle length after treatment in this monkey was identical to that before treatment.

In Figure 3, the typical pattern of estrogen and progesterone in plasma is illustrated in one of the monkeys treated with 1 mg lynestrenol daily. As can be seen ovulations occurred in the control and recovery cycles. During the treatment cycle there were no indications of the occurrence of ovulation as evidenced by the low progesterone levels (below 0.4 ng/ml) in plasma in the second half of the cycle.

Anovulatory cycles were observed in four of the five monkeys receiving 2.5 mg lynestrenol per day. The effect of the treatment cannot be judged in the fifth monkey due to the onset of summer amenorrhea. The treatment cycle lengths in three of these monkeys were five to nine days longer than the control cycle lengths.

The results of the measurement of the steroids in a monkey before and during the cycle in which 2.5 mg lynestrenol per day was given, are shown in Figure 4. The steroid pattern was very uniform in all the monkeys treated with 2.5 mg of lynestrenol. The estrogen levels in plasma were low throughout the treatment cycles and even decreased to levels below the lowest levels in the control cycles. The progesterone levels in plasma were at or below the practical detection limit of the method which means below 0.2 ng per ml plasma. It was obvious from the steroid pattern in this and the other three monkeys treated with 2.5 mg lynestrenol that all the treatment cycles were anovulatory.

Plasma levels of norethindrone approximately 24 hours after the intake of the 1 mg lynestrenol tablets varied between 0.09 – 1.35 ng per ml plasma with a mean of 0.39 ng per ml. In the monkeys receiving 2.5 mg of lynestrenol a range of 0.08 – 5.75 and a mean of 2.26 ng per ml of plasma were found. Measureable quantities were found in all samples during treatment but not during the control cycles.

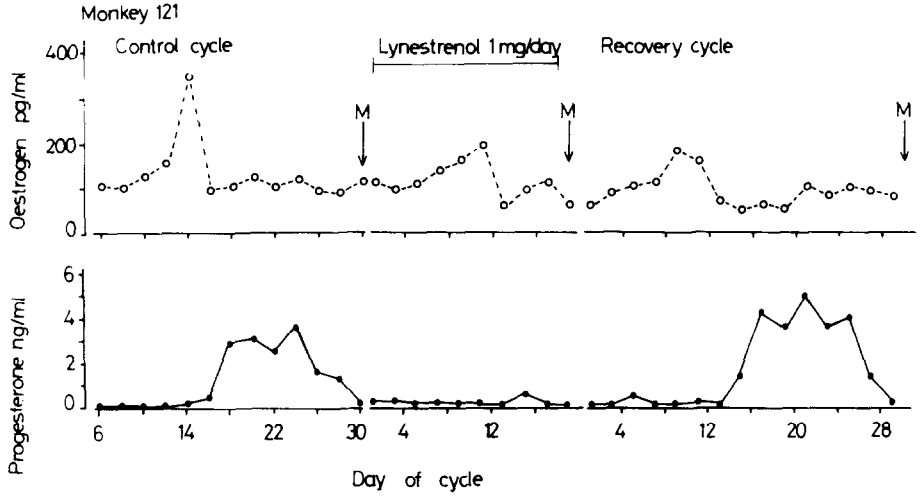

Fig. 3. Peripheral plasma levels of oestrogen and progesterone during three consecutive menstrual cycles in a rhesus monkey. Lynestrenol 1 mg/day was given orally throughout the second cycle.

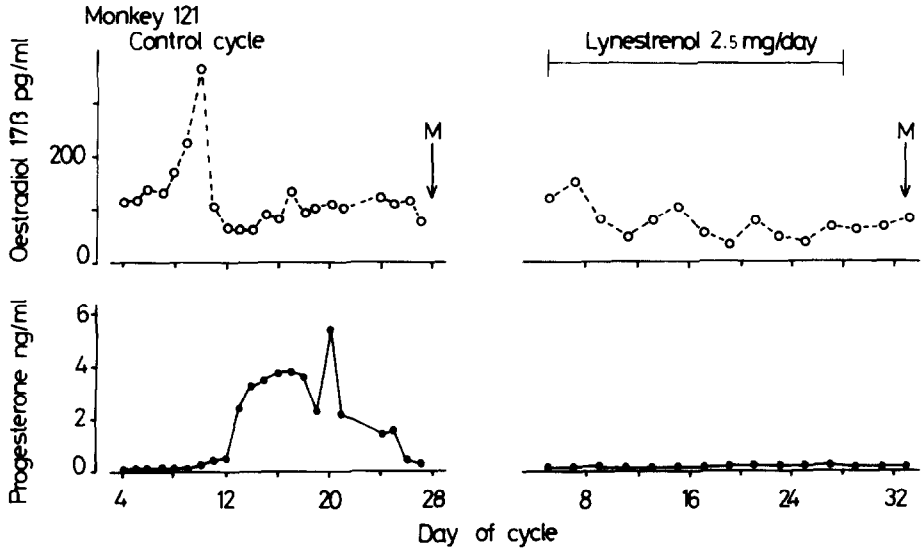

Fig. 4. Peripheral plasma levels of oestrogen and progesterone in a rhesus monkey during a control cycle and the following cycle when lynestrenol 2.5 mg/day was administered orally from day 4 – 26.

The compound was well tolerated at all dose levels used. In some of the monkeys treated with 1 and 2.5 mg lynestrenol slight leucorrhoea was observed.

DISCUSSION

According to the results of measurements of estrogens and progesterone in the monkeys treated with 0.05 and 0.25 mg lynestrenol, it appears that lynestrenol at these dose levels does not inhibit ovulation in rhesus monkeys. At dose levels of 1 and 2.5 mg per day throughout the menstrual cycle, lynestrenol appears to prevent ovulation in the rhesus monkeys. It was also apparent from the steroid patterns that at 1 mg levels, lynestrenol had little effect on the estrogen levels recorded during the first half of the cycles while treatment with 2.5 mg lynestrenol appears to result in low levels of estrogens throughout the cycle.

In order to ascertain that the monkeys really swallowed and absorbed the lynestrenol, the plasmas from monkeys receiving 1.0 and 2.5 mg were assayed by a radioimmunoassay system that detects norethindrone and its unconjugated metabolites (5). According to Fotherby (10) lynestrenol is metabolized via norethindrone and is likely to exert its biological effect through norethindrone at least in women. The levels of norethindrone found indicated that the monkeys swallowed all their pills. Unfortunately the study was started before the radioimmunoassay for norethindrone was available. Neither the time of tablet intake or the exact time of sampling was recorded.

The site of action of lynestrenol, a progestogen with inherent estrogenic activity, when given to women was supposed to be at the hypothalamo-pituitary axis resulting in the depression of the FSH and LH production during the menstrual cycle (11). The female rhesus monkeys weigh approximately one tenth of a woman but appears to require about the same daily dose as women for inhibition of ovulation which means ten times more per kg body weight.

On the basis of the present findings it would appear that at the 1 mg dose level, lynestrenol inhibits ovulation in the monkeys through interfering with estrogen induced LH surge or action. On the other hand, 2.5 mg lynestrenol per day appears also to inhibit follicular maturation and the base line production of estrogens. Both types of action have been recorded for progesterone in the rhesus monkey (12, 13, 14). In women ovulation is inhibited by plasma levels of norethindrone at and above 1 ng per ml (15). Despite the inaccuracy in timing of the blood samples, the levels of norethindrone found indicates that approximately the same plasma levels of norethindrone are required for inhibition of ovulation in rhesus monkeys as in women.

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