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Pentobarbital and ketamine suppress serum concentrations of sex hormones in the female rat

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Abstract
Purpose. To investigate the potential effects of pentobarbital and ketamine on serum concentrations of sex hormones, the present study was performed using pregnant mare serum gonadotropin (PMSG)-primed cyclic female Sprague-Dawley rats.
Methods. Pentobarbital sodium (37 mg·kg^{-1}, i. p.) or ketamine-hydrochloride (229 mg·kg^{-1}, i. m.) was injected 2 and 3 days after PMSG treatment. At 0, 1, 2, 3, 4, and 5 days after PMSG treatment, sera were collected by cardiac puncture. The serum concentrations of progesterone (P_4), testosterone (T), and estradiol-17β (E_2) were determined by radioimmunoassay.
Results. The serum concentrations of P_4 tended to be lower in both the pentobarbital- and the ketamine-treated groups compared with the control group. Significant differences were found on days 3 and 4 after pentobarbital and on days, 1, 4, and 5 after ketamine administration. Serum concentrations of T were also suppressed in both the pentobarbital and the ketamine-treated groups, whereas E_2 concentrations decreased only in the ketamine-treated group.
Conclusion. Pentobarbital and ketamine decrease serum sex hormone concentrations in PMSG-primed female rats.

Key words Pentobarbital · Ketamine · Sex hormones

Introduction

Ovarian steroidogenesis is under endocrine control by the hypothalamic–pituitary–ovarian axis [1]. Follicle-stimulating hormone (FSH) increases the number of granulosa cells (GC), the number of FSH receptors on GC, and the diameter of follicles. FSH also increases the activity of aromatase, the number of luteinizing hormone (LH) receptors, and the number of growing follicles and decreases the atresia of antral follicles in rodents [2,3]. Pregnant mare serum gonadotropin (PMSG) has been used to induce the development of ovarian follicles in rodents.

Pentobarbital suppresses the afternoon surge of LH [4], FSH, prolactin, progesterone, and plasma estradiol [5,6], and induces atresia of preovulatory follicles [7,8]. It is unclear, however, whether the induction of follicular atresia by anesthetics is initiated by the blockage of LH surge or by a direct effect on the ovary. Similarly, ketamine has a genotoxic effect [9]. The ovulation rate varies with the time of anesthesia after treatment with human chorionic gonadotropin [10]. In other reports, however, the ovulation rate did not change after ketamine treatment during the proestrus period [11]. Ketamine suppressed the ovulatory LH surge in females and decreased the baseline LH level in males [12].

On the other hand, it remains unclear whether there is a direct action of anesthetics on the ovary. Therefore, we studied the putative ovarian toxicities of pentobarbital and ketamine by determining the serum concentrations of sex hormones.

Materials and methods

Twelve-week-old female Sprague-Dawley rats were maintained in groups of five per cage in a 25°C temperature-controlled animal care room with a light/dark cycle (14/10 h) in Hanyang University, Seoul, Korea. The animals had free access to tap water and commercial chow during experiments.

Pentobarbital (37 mg·kg^{-1}, Nembutal, Abbott Laboratories, North Chicago, IL, USA) was injected intraperitoneally between 1200 and 1300 h 2 days after 10 i.u. of PMSG (Sigma) injection according to the method of van der Schoot [13]. The dose was approximately 28% of the lethal dose (LD_{28}). Ketamine

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(229 mg·kg^{-1} Ketanest, Parke-Davis, Munich, Germany) was injected intramuscularly according to the manufacturer's prescription at LD_{50}. Control animals were injected with saline. Serum was collected at 0, 1, 2, 3, 4, and 5 days after 48 h of PMSG injection. Estrous cycles were monitored daily by examination of vaginal cytology. The number of rats in each group was 5 to 10 (Fig. 1).

Concentrations of serum progesterone (P_4), testosterone (T), and estradiol-17β (E_2) were determined by radioimmunoassay as described by Yoon et al. [14]. Antisera of P_4, T, and E_2 were raised with P_4-2-carboxymethyl oxime (CMO)-bovine serum albumin (BSA) (titer, 1/35 000), T-3-CMO-BSA (titer, 1/84 000), and E_2-6-CMO-BSA (titer, 1/10 500), respectively. The intra- and inter-assay coefficients of P_4, T, and E_2 were 17.4% and 9.6%, 10.4% and 7.8%, and 9.5% and 18.4%, respectively. The lower detection limits of P_4, T, and E_2 were 150, 10, and 20 pg, respectively.

Data are presented as means ± SEM and were statistically analyzed by analysis of variance and Duncan's multiple range test. Differences are considered to be significant at $P < 0.05$.

Results

PMSG-primed modulation of P_4, T and E_2 synthesis was recognized in the saline-injected control group. A cyclic pattern of steroid secretion was shown in control animals (Table 1). The serum concentration of P_4 gradually increased until it reached its highest value at 4 days after 48 h of PMSG injection, then returned toward its normal value.

Pentobarbital and ketamine decreased serum concentrations of P_4. The concentrations of P_4 decreased by about 50% at 3 days and by more than 60% at 4 days in pentobarbital-treated rat serum. Serum P_4 concentrations were also decreased at 1, 4, and 5 days in ketamine-treated rat serum (Fig. 2).

The serum concentration of T was also decreased by treatment with pentobarbital and ketamine (Fig. 3). At 2–3 days, the serum concentration of T was significantly decreased in the ketamine-treated group. In the pentobarbital-treated group, the serum concentration of T was significantly decreased 2–5 days after 48 h of PMSG injection.

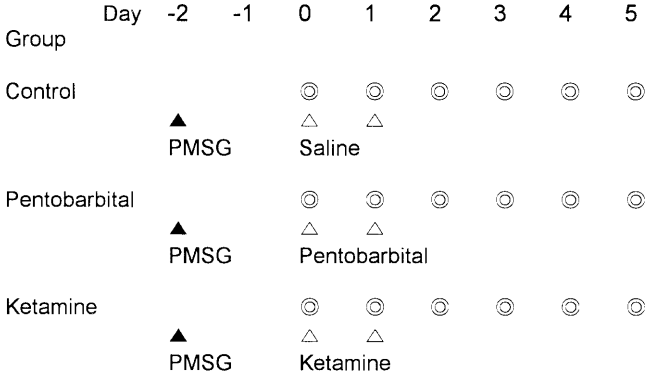

Fig. 1. Time schedule for treatments and sampling. On day −2, pregnant mare serum gonadotropin (PMSG, 10 i.u., ▲) was injected i. p. to synchronize the estrous cycle. Saline, pentobarbital (37 mg·kg^{-1}, i. p.), or ketamine (229 mg·kg^{-1}, i. m.) was injected on days 0 and 1 (△) after PMSG administration. All the injections and samplings were carried out between 1200 and 1300 on each day. ◎, Sacrifice dates

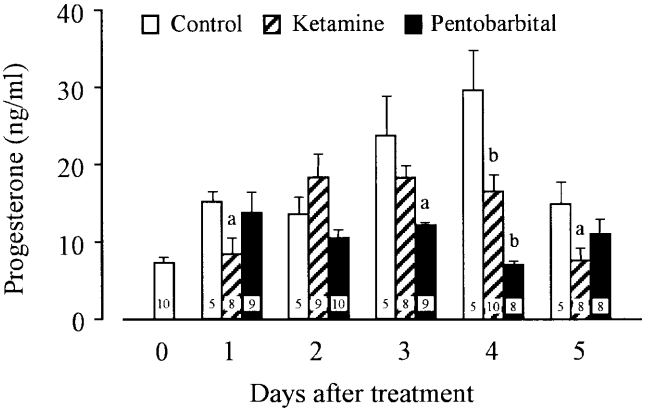

Fig. 2. Serum concentrations of progesterone after treatment with saline, pentobarbital, and ketamine in PMSG-primed cyclic rats. *a*, $P < 0.05$; *b*, $P < 0.01$, significantly different from the control group. Data are expressed as means ± SEM. The number of rats is indicated in each bar

Table 1. Serum steroid concentrations in normal cyclic rats[a]

Stage of cycle	Progesterone (ng·ml^{-1})	Testosterone (ng·ml^{-1})	Estradiol (pg·ml^{-1})
Proestrus	10.54 ± 0.03 (10)	0.64 ± 0.09 (6)	9.12 ± 2.18 (5)
Metestrus	6.04 ± 0.34 (9)	0.62 ± 0.06 (7)	11.29 ± 3.38 (5)
Diestrus	12.52 ± 0.88 (10)	1.01 ± 0.05 (9)	23.47 ± 3.77 (5)
Estrus	9.96 ± 0.91 (7)	0.66 ± 0.04 (8)	13.21 ± 2.32 (5)

[a] The concentrations of steroid hormones were determined by radioimmunoassay and expressed as means ± SEM. The lower detection limits of the concentrations were 150, 10, and 20 pg for progesterone, testosterone, and estradiol, respectively. The number of rats in each group is indicated in parentheses.

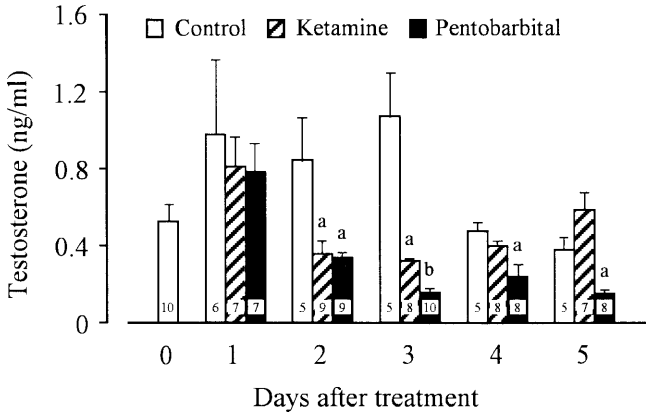

Fig. 3. Serum concentrations of testosterone after treatment with saline, pentobarbital, and ketamine in PMSG-primed cyclic rats. *a*, $P < 0.05$; *b*, $P < 0.01$, significantly different from the control group. Data are expressed as means ± SEM. The number of rats is indicated in each bar

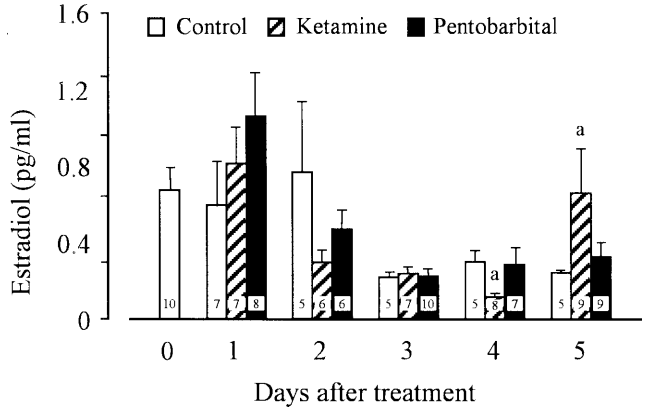

Fig. 4. Serum concentrations of estradiol-17β after treatment with saline, pentobarbital, and ketamine in PMSG-primed cyclic rats. *a*, $P < 0.05$, significantly different from the control group. Data are expressed as means ± SEM. The number of rats is indicated in each bar

The serum concentration of E_2 was not significantly affected by treatment with pentobarbital. However, the serum concentration of E_2 was decreased at 2 and 4 days and increased at 5 days in the ketamine-treated group (Fig. 4).

Discussion

Generally, PMSG has been used for the induction of superovulation. It has the actions of both FSH and LH. In the present experiments, we observed the PMSG-primed modulation of serum P_4, T, and E_2 concentrations by pentobarbital and ketamine. The cyclic patterns of these steroid secretions in saline-injected control animals agreed with the well-established pattern in the concentrations of steroid hormones [15].

During culture of follicles isolated from pentobarbital-treated rats, the secretion of estrogen and androgen was significantly reduced and P_4 production remained unchanged [8]. Serum E_2 concentrations on day 1 were lower than those during proestrus in ovulation-blocked rats [15,16]. In accordance with the reports by Barraclough et al. [6] and Uilenbroek et al. [8], the concentrations of P_4 and T were decreased in all groups in the present study. On day 5, the serum E_2 concentration was increased in the ketamine-treated rats. This finding is different from those of previous studies [15,16] and shows that these are some differences between pentobarbital and ketamine in the effect on E_2 synthesis. In normal rats, the ovaries secrete predominantly E_2 before ovulation and produce mainly progesterone after ovulation [17]. Mizuno et al. [18] reported that ovulation in rats was delayed by 24 h by the administration of pentobarbital early in the afternoon of proestrus. We administered the anesthetics twice at 1 and 2 days to block ovulation completely.

Although ketamine had no effect on ovulation [13] and on serum FSH, LH, and prolactin levels [5], it caused decreases in serum P_4 and T concentrations. This finding indicates that there is a direct effect of ketamine on the ovary. In addition, the serum concentrations of P_4 and E_2 on day 0 were not the same as those during proestrus. It is assumed that the differences between experimental and control animals were due to the effect of the exogenous PMSG administered 2 days previously.

The present study revealed that pentobarbital and ketamine suppressed P_4 and T production in vivo. However, the possibility of direct cytotoxic effects of these anesthetics on the ovary should be further evaluated. We observed that pentobarbital and ketamine were similar in reducing the serum levels of steroid hormones in the cyclic female rat, with the exception of E_2 production. It remains unclear whether the use of these anesthetics in clinical settings might impair female reproductive organs, including the ovary.

In conclusion, treatment with pentobarbital or ketamine suppresses the serum concentrations of sex hormones in PMSG-primed female rats by direct action of the anesthetics on the ovary.

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