Time-Dependent Ovulation-Blocking Effect of Ether Anesthesia Differs from Pentobarbital in Rats

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KIM, C.-Y., WAKABAYASHI, K. and NOBUNAGA, T. Time-Dependent Ovulation-Blocking Effect of Ether Anesthesia Differs from Pentobarbital in Rats. Tohoku J. Exp. Med., 1994, 172 (3), 237-242 —— Time-dependent ovulation-blocking effect of ether anesthesia was investigated using female Wistar-Imamichi rats showing a 4-day cycling rhythm. A 6-hr (1400-2000) and 8-hr (1400-2200) anesthesia with ether vapor on proestrus caused ovulation blockade in 85% and 100% of the rats, respectively. On the other hand, pentobarbital injections at 1400 and 1500 completely blocked the spontaneous ovulation, while the injections at 1600 and 1700 did not. Ether (1400-2000) and pentobarbital anesthesia decreased the mean serum LH levels at 2000 on proestrus. The LH levels were 13.26 ng/ml for the control, 0.36 ng/ml for the ether group and 0.56 ng/ml for the pentobarbital group, respectively. These findings indicated that ether and pentobarbital similarly suppressed the LH surge, but difference between ovulation-blocking effects by ether and pentobarbital is possibly due to factor(s) other than suppression of the LH surge. —— ether anesthesia; pentobarbital; critical period; lighting condition; ovulation

It has been well known that various factors affect ovulation. Among others lighting conditions (Takahashi and Suzuki 1969) and anesthetics have been extensively studied (Dickmann and Terranova 1990). Injection of anesthetics, such as urethane, phenobarbital or pentobarbital, in the afternoon of the proestrus day inhibited the spontaneous rise of plasma luteinizing hormone (LH) levels and the delay of ovulation was observed (Blake and Sawyer 1972; Blake 1974). Prolonged exposure to ether vapor also inhibited the rise of plasma LH. However, short exposure to ether increased circulating levels of prolactin in male rats (Wakabayashi et al. 1971; Krulich et al. 1974; Chi and Shin 1978; Mattheij

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Ether anesthesia even induced a rapid release of follicular stimulating hormone (FSH) and LH during various stages of estrous cycle, except at the afternoon of proestrus (Basha et al. 1984). In the afternoon of proestrus exists "critical period", the delimited period all the while administration of the various drugs prevent ovulation (Everett 1956).

In many literature on endocrine investigations as well as the reports mentioned above have been adopted a 14 hr light and 10 hr dark cycle (light from 0500 to 1900). Recently it appears that a 12 hr light and 12 hr dark lighting condition, such as lighting period from 0800 to 2000, is adopted in institutes for experimental animals to lengthen available and convenient time for work.

Therefore, in the present study the period of ether anesthesia being effective to inhibit ovulation in rats is determined under the lighting condition of 12 hr light and 12 hr darkness with lighting period from 0800 to 2000. Time-dependent ovulation-blocking effects of ether and pentobarbital are compared and the effect of pentobarbital is precisely examined to estimste the critical period under the lighting condition above.

**MATERIAL AND METHODS**

*Animals and observation of vaginal smear*

Female Wistar-Imamichi strain rats, maintained in the Institute for Experimental Animals, Tohoku University School of Medicine, were used at 12 weeks of their age. They were kept under the controlled lighting condition of 12 hr light and 12 hr darkness with lighting period from 0800 to 2000. The room temperature and humidity were adjusted at 24±1°C and 50-60%, respectively. Rat chow (F1, Funabashi Farm Co. Ltd., Chiba) and water were available at all times. Their vaginal smears were taken at 1130 and observed under a microscope. Then they were classified into estrus, metestrus, diestrus, or proestrus according to the criterion established by Nobunaga and Nakamura (1968). After confirming regular 4-day cyclicity for 2 weeks, they were used for the following experiments.

*Exposure to anesthetics*

A group of rats were anesthetized with ether vapor in the apparatus shown in Fig. 1. The exposure chamber consists of a 7.5 liter glass deccicator with air flow of 1.5 liter/min. The chamber was connected to the ether vapor generator. Ether (special grade diethyl ether, Wako Pure Chemical Industries LTD., Osaka) was vaporized by aeration and diluted with fresh air at a ratio of approximately 1:6. The room was controlled at 24±1°C throughout the exposure.

In each trial, one or two rats in the stage of proestrus were placed in the chamber for exposure to ether vapor. Each rat was exposed for one of the three periods; 1400-1700, 1400-2000, or 1400-2200.

Time-dependent blocking effects of spontaneous ovulation was examined in groups of rats injected i.p. with 40 mg/kg of pentobarbital (Nembutal, Abbot Laboratories, North Chicago, IL, USA). The time of the injections were 1400, 1500, 1600, or 1700, respectively. The rats of the control group for the anesthetics were injected with saline as well.

*Examination of ovulation*

Ovulation was examined as follows: After being sacrificed by cervical dislocation, uterus, uterine fluid and ovaries were collected. Both oviducts were removed from the
uterus, and placed on a glass slide with a drop of saline. The oviducts were covered with another slide and squeezed with both slides being gently pressed. Each ovum found in the distended ampulla was counted under a dissecting microscope.

Ovulation was examined at 0930 for all the groups with various treatments. However, for the control group with saline, it was also examined at 0300, 0400 and 0600.

**Determination of serum luteinizing hormone levels**

To determine serum LH levels rats were decapitated at 2000 on the day of proestrus after administration of saline, pentobarbital (30 mg/kg i.p.) or exposure to ether vapor for 6 hr (1400–2000), respectively. Blood was drawn from the trunk and placed into centrifuge tubes. Clotting was allowed to occur. The sera were separated by centrifugation at 4°C for 15 min. The serum samples were stored frozen at −80°C until assay.

LH was determined by radioimmunoassay using NIDDK RIA kit supplied by the National Hormone and Pituitary Program. The kit consists of NIDDK rat LH I-9, NIDDK anti-rat LH S-10, and NIDDK rat LH RP-3. Radioimmunoassay was carried out by double antibody method according to the instruction by NIDDK with minor modification; i.e., goat antirabbit IgG serum (Hormone Assay Center, Institute of Endocrinology, Gunma University) was used as the second antibody. An excellent standard curve was obtained with RP-3 from 0.25 to 32 ng/ml, and within- and between-assay coefficients of variation were 5.3%, and 8.6%, respectively.

**Statistical analysis**

Statistical analyses were performed by one-way analysis of variance and significantly different groups were identified using the Duncan's multiple range test.

**Results**

**Effects of ether anesthesia and pentobarbital on ovulation**

As expected, all the control rats ovulated in the next morning of the treatment (Day 1). Precise observation of ovulation (data not shown in the table) revealed that the control (non anesthetized rats) ovulated none out of 6 at 0300, 5 out of 12 at 0400, and 6 out of 6 at 0600, respectively. Therefore, it was considered to be valid to observe ovulation at 0930 under the lighting condition used. None of the rats injected with pentobarbital at 1400 ovulated (Table 1).
Blockade of ovulation was also confirmed in the group 2 (5 out of 7) or 3 (4 out of 4), given ether anesthesia between 1400-2000 or 1400-2200 of the proestrous day. However, in the group 1, with a shorter anesthesia period (1400-1700), ovulation was not blocked. The next day (Day 2) all the rats anesthetized with pentobarbital or the two regimens of ether exposure showed ovulation.

**Changes in serum LH level by ether anesthesia and pentobarbital**

LH levels determined at 2000 are shown in Table 2. The mean serum LH level in the control was 13.26 ng/ml, while that in the groups treated with pentobarbital and ether (1400-2000) decreased significantly. The difference between the two treated groups was not statistically significant.

**Effects of pentobarbital injections at different time on ovulation**

Pentobarbital injections at 1400 and 1500 induced complete suppression of ovulation at 0930 the next morning (Table 3). However, 2 out of 7 rats ovulated when the injections were performed at 1600 or 1700. These results suggest that

<table>
<thead>
<tr>
<th>Table 1. Ovulation after ether anesthesia and pentobarbital injection</th>
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<tr>
<td><strong>Day 1</strong></td>
</tr>
<tr>
<td>Ovulating /total</td>
</tr>
<tr>
<td>Ether anesthesia</td>
</tr>
<tr>
<td>Group 1 (1400-1700)</td>
</tr>
<tr>
<td>Group 2 (1400-2000)</td>
</tr>
<tr>
<td>Group 3 (1400-2200)</td>
</tr>
<tr>
<td>Pentobarbital</td>
</tr>
<tr>
<td>Control (Saline)</td>
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</table>

* the expected estrous day  
* the expected metesrous day  
* number of rats ovulated/total number of rats in each treatment  
* mean number of ova with s.d.

<table>
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<tr>
<th>Table 2. Effects of ether and pentobarbital anesthesia on the serum LH concentrations*</th>
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<tbody>
<tr>
<td>Ether anesthesia (1400-2000)</td>
</tr>
<tr>
<td>LH ng/ml</td>
</tr>
</tbody>
</table>

*Animals were sacrificed at 2000 on the proestrous day after anesthesia starting at 1400.  
bValues represent the means±s.d.

One-way ANOVA was highly significant, but the Duncan’s multiple range test revealed no significant difference between ether and pentobarbital anesthesia.
The present study clearly demonstrates inhibition of ovulation by ether anesthesia and pentobarbital injection. However, the effects of anesthetics were different in terms of time factor; the length and timing of injections. Complete inhibition of ovulation was observed for the ether anesthesia only in the group 3, which were anesthetized between 1400-2200. These findings are basically similar to the report of Blake (1974) who demonstrated that ether anesthesia longer than 6 hr was to be maintained to inhibit spontaneous rise of LH.

As for the pentobarbital treated rats injections at 1400 and 1500 on the proestrous day were completely effective in inhibiting ovulation. LH determination revealed both the anesthetics caused suppression of LH rise and consequently it was likely that rats did not ovulate in the expected estrous morning.

The groups of rats, which did not ovulate in the expected morning, ovulated later on Day 2. Therefore, effects of both the anesthetics were transient and not permanent. Numbers of ova in these groups of rats which ovulated later were not different from the control. This finding also suggests the effects were transient.

While the determined LH levels were similar between ether anesthesia and pentobarbital, the patterns of inhibiting ovulation were different in terms of completely effective regimens. Among the ether anesthesia, complete suppression of ovulation required anesthesia covering the period of 1400-2200. The shorter two regimens were not enough to block the ovulation completely, even though the exposure started at the same time (1400) for each anesthesia. On the other hand, pentobarbital injections at 1400 and 1500 completely blocked the spontaneous ovulation, but the injections at 1600 and 1700 did not.

Therefore, it is likely that the ovulation blocking mechanism by ether is not same as that by pentobarbital. Since ovulation occurs as the consequence of multi-step processes, the LH surge, presumably, is a step of the cascades. Thus, ether and pentobarbital similarly suppressed the LH surge as observed in the present experiment and the difference between ether and pentobarbital in block-

**Table 3. Time-dependent effects of pentobarbital on subsequent ovulation**

<table>
<thead>
<tr>
<th>Pentobarbital injection</th>
<th>Ovulating/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 1400</td>
<td>0/11</td>
</tr>
<tr>
<td>at 1500</td>
<td>0/7</td>
</tr>
<tr>
<td>at 1600</td>
<td>2/7</td>
</tr>
<tr>
<td>at 1700</td>
<td>2/7</td>
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</tbody>
</table>

*Animals under lighting condition (0800-2000) were sacrificed on the expected estrous morning (0930) after administration of pentobarbital (40 mg/kg i.p.) at different time of the proestrous afternoon.

*Number of rats ovulated/total number of rats in each treatment group.
ade of ovulation is possibly to be based on factor(s) other than suppression of the LH surge.

The beginning of the critical period was not confirmed in the present study. However, pentobarbital injections at 1400 and 1500 were effective completely to block ovulation. Therefore, the critical period started at not earlier than 1400. Moreover, the end of critical period was considered to be around 1600 for the individual rats probably with the shorter period.

The shorter anesthesia periods with ether did not produce complete inhibition of ovulation. Unidentified event that occurred before 2200 may cause subsequent ovulation. Further study is necessary to elucidate the differential effects between ether anesthesia and pentobarbital on blocking ovulation.

Acknowledgment

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References


