Effect of Pentobarbital and Ether Stress on Serum Prolactin Levels in Rats[1] (35753)

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Ether and pentobarbital are the most common anesthetic agents employed in laboratory experiments involving animals. These anesthetics are frequently used when blood is withdrawn from the animals. Ether inhalation is also employed as a stressful stimulus to experimental animals.

Ether, as well as other stressful stimuli, is known to increase serum ACTH levels but reduce plasma immunoreactive GH in rats (1). Ether is administered when blood is withdrawn during a study of prolactin secretion (2). Ether itself, as well as other nonspecific stressful stimuli, could influence serum prolactin levels. It is also possible that the magnitude or pattern of responses to ether inhalation might differ between sexes. Similar problems may exist when pentobarbital is used at the time of blood collection. Since this drug has a biphasic effect (stimulatory and suppressive) in ACTH release (3), the effect of administration of pentobarbital on prolactin secretion could also vary depending on the dose, time of administration, or sex of the animal. Information about these problems may be important when secretory patterns of prolactin are investigated in animals under ether or pentobarbital anesthesia. The studies presented here investigate the effects of pentobarbital administration, ether stress, and preexperimental handling on serum prolactin levels in male and female rats.

Materials and Methods. Young adult female and male rats of the Sprague-Dawley strain, 180-200 g of body wt (Cheek Jones Farm, Houston, Texas), were used throughout the experiments. These animals were housed in group cages with constant temperature (78 ± 1°F) and 12 hr of light/day. They were fed Purina Lab. Chow, supplemented with fresh vegetables and water *ad libitum*. Some of these animals were gentled by handling twice daily for 4 days before the experiments. Some rats were injected with pentobarbital sodium (Nembutal, Abbott Lab., North Chicago, Ill.). The control rats were injected with saline. Some were exposed to ether vapor in a jar for 1 min. To avoid unnecessary stimuli to the rats, all investigators and technicians, when they handled the animals, wore gloves which were exclusively used for handling rats. In another room, blood was collected from the trunk after decapitation. This was completed 30 to 60 sec after the animal was removed from the cage. Caution was taken to minimize various nonspecific stressful effects on the experimental animals. Serum was separated by centrifugation and kept frozen until assayed for prolactin. The stages of estrous cycle in female rats were disregarded, but to avoid the influence of possible diural fluctuations in responses, all experiments were done between 9 a.m. and 11 a.m. Serum prolactin was measured by radioimmunoassay method developed by Niswender *et al.* (4) with slight modifications, using the NIAMD rat prolactin radioimmunoassay kit. All samples were measured in duplicate at two dilution levels. The mean of these values was considered as the final value of prolactin level. Comparison between mean values in various treatment groups was made according to Duncan's new multiple range test. To test the effects of the interaction between sexes, handling and nonhandling, ether and pentobarbital, $2 \times 2 \times 3$ analysis of variance was employed (5).

Results. As shown in Table I, the mean

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TABLE I. Serum Prolactin Levels (ng/ml) in Rats Under Various Conditions.

Rats	Group no.	Intact	Group no.	Ether[c]	Group no.	Pentobarbital, 3.5 mg/100g[d] [e]	p Value
Male							
Non handled	1	7 ± 3.9[a] (5)[b]	2	22 ± 2.1 (5)	3	47 ± 12.7 (5)	1 vs 2: .05 1 vs 3: .05
Handled	4	15 ± 3.8 (5)	5	37 ± 5.7 (5)	6	42 ± 9.9 (5)	4 vs 5: .05 4 vs 6: .05
Female							
Non handled	7	27 ± 4.9 (5)	8	26 ± 2.6 (5)	9	277 ± 58.3 (5)	7 vs 8: NS 7 vs 9: .01
Handled	10	13 ± 4.5 (5)	11	51 ± 11.7 (5)	12	278 ± 38.8 (5)	10 vs 11: .05 10 vs 12: .01

[a] Mean ± Standard error.
[b] Number of animals.
[c] Five min after ether stress.
[d] Thirty min after injection.
[e] 2 × 2 × 3 analysis of variance indicated that there was a significant interaction between effects of pentobarbital and sex of animals.

resting levels of serum prolactin in nonhandled female rats was higher than that of male rats ($p<.05$). Handling for 4 days lowered prolactin levels in female rats, but raised them in male rats. It was noticed that female rats became accustomed to handling within 4 days as indicated by relaxation of muscle tonus when they were handled, whereas male rats became alert and appeared to be apprehensive after 4 days of handling.

Five min after ether inhalation, serum prolactin levels increased two to four times over corresponding resting levels in all groups except the nonhandled female rats. When the t test was used to compare the ether-stressed rats with the corresponding intact rats, the difference in mean prolactin levels between these two groups was highly significant. In later experiments, it was found that serum prolactin reached the peak within 2.5 min after ether stress and returned to resting levels 15 min after stress (Fig. 1).

Injection of 3.5 mg of pentobarbital/100 g of body wt ip significantly increased serum prolactin levels in all groups after 30 min as compared with corresponding resting levels, but a greater increase of serum prolactin was observed in female rats (Table I). Analysis of variance indicated that the magnitude of responses to pentobarbital between sexes is significantly different ($p<0.01$). In another experiment with male rats, the same dose of pentobarbital increased serum prolactin only slightly 15 min after the injection. Prolactin levels returned to the preinjection levels 30 and 60 min after the injection (Fig. 2). Since injection of saline also resulted in a slight increase in serum prolactin levels, the effect of injection of 3.5 mg of pentobarbital on serum prolactin in male rats may not be specific. In female rats, 3.5 mg of pentobarbital/100 g of body wt significantly raised serum prolactin levels over the entire 60-min period compared with saline-injected controls. The peak responses were observed around 30 min after the injection (Fig. 2). It was noticed that female rats were completely anesthetized with 3.5 mg of pentobarbital/100 g of body wt, while most of the male rats which received the same dose reflexly increased their muscle tonus when they were removed from the cage, indicating that the depth of anesthesia was shallow. In male rats, 5 mg of pentobarbital/100 g of body wt was needed to obtain complete anesthesia and this resulted in a significant increase of serum prolactin levels compared with those in saline-injected controls. The pattern of changes in serum prolactin levels after injection of 5 mg of pentobarbital/100 g of body wt was similar to that obtained in female rats with 3.5 mg of pentobarbital/100 g of body

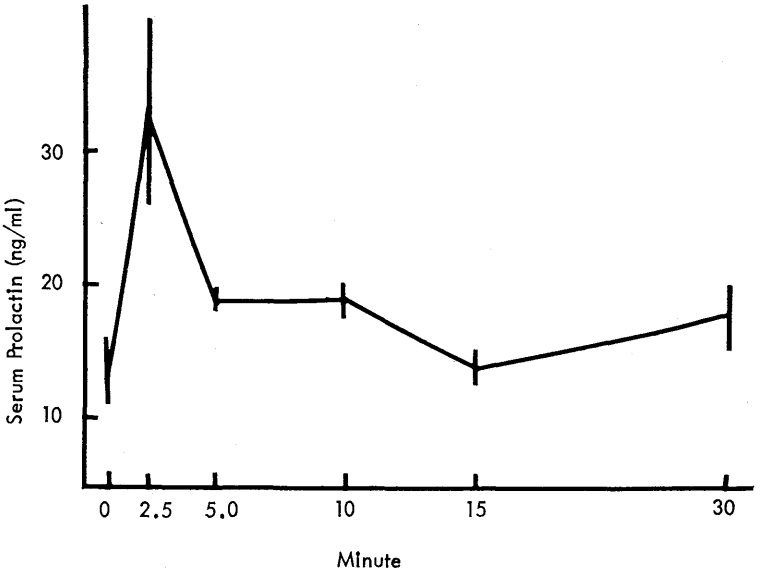

FIG. 1. Serum prolactin levels after ether inhalation in nonhandled male rats: 4–5 rats were used for each point; vertical bars indicate standard error of the mean.

wt.

Discussion. Injection of 3.5 mg of pentobarbital/100 g of body wt 30 min previously increased serum prolactin levels in female rats compared with resting levels, but the extent of increase was very slight in male rats. In another experiment, the same dose of pentobarbital resulted in a significant increase in serum prolactin over the entire 60-min period in female rats. Male rats showed only a slight increase 15 min after the injection of pentobarbital; a similar rise of serum prolactin was also seen 15 min after saline injection. Female rats were anesthetized more deeply than male rats with 3.5 mg of pentobarbital/100 g of body wt. In male rats, 5 mg of pentobarbital/100 g of body wt was needed to obtain results similar to those seen in female rats with 3.5 mg of pentobarbital/100 g of body wt. When a sufficient dose of pentobarbital was administered, in both male and female rats serum prolactin levels reached a peak around 30 min after the injection and began decreasing thereafter. The magnitude of elevation of serum prolactin levels seemed to be correlated with the depth of anesthesia; the deeper the stage of anesthesia, the more prolactin was released.

It is generally accepted that the release of prolactin is regulated by the hypothalamic prolactin-inhibiting factor (PIF) (6). Various tranquilizing agents such as phenothiazine and reserpine have been reported to suppress PIF activity in the hypothalamus, thereby increasing serum prolactin levels (6). For the present, it is reasonable to assume that an increase of serum prolactin levels by pentobarbital is mediated also by suppressing endogenous PIF, as in the case of the above drugs.

The rise in serum prolactin after 1 min of ether inhalation was quite rapid and short in duration (Fig. 1). The pattern of prolactin changes after ether inhalation is similar to those of ACTH (7) and MSH (8) after ether stress. MSH release, like prolactin release, is also controlled primarily by a hypothalamic inhibitor (9). Grosvenor *et al.* (10) suggested that the existence of prolactin-releasing factor (PRF) was necessary to explain the rapid discharge of prolactin following suckling stimulus in lactating rats. Recently, the possible existence of PRF in rat hypothalamus was reported by Nicoll *et al.* (11). Although the increase of serum prolactin following ether stress could be mediated by acute suppression of hypothalamic

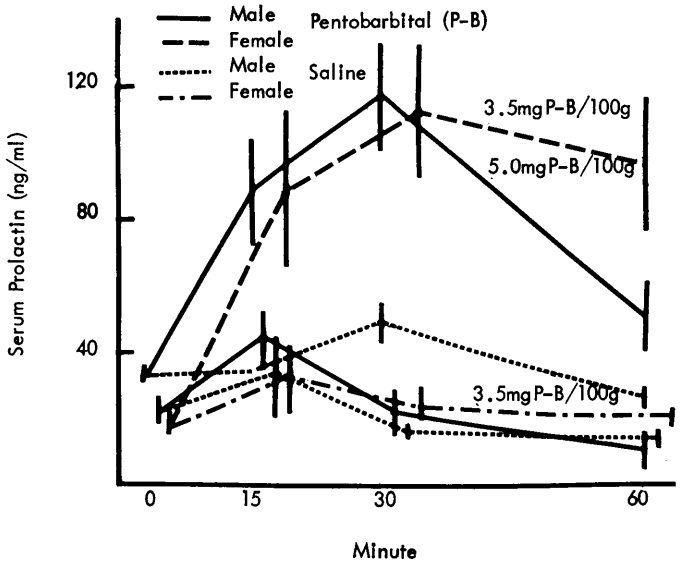

FIG. 2. Serum prolactin levels after pentobarbital injection in nonhandled male and female rats: 5–6 rats were used for each point; vertical bars indicate standard error of the mean.

PIF, the response could be also induced by the release of PRF. A classical experiment by Everett and Quinn (12) showed that electric stimulation of the hypothalamus stimulated prolactin release and not the inhibition of release.

Resting levels of serum prolactin in handled female rats were lower than those of nonhandled. Opposite results were obtained in male rats, which became more alert after handling. A period of 4 days of handling was not long enough to allow these male animals to become accustomed to manipulation. Handling of male rats in this experiment may have acted as a stressful stimulus rather than a gentling procedure and resulted in increased prolactin release.

Amenomori *et al.* (2) reported that resting serum prolactin levels in normal adult female rats showed cyclic changes during the estrous cycle. It is well known that ovarian steroids affect the mechanisms regulating prolactin release either at the pituitary or at the hypothalamic level. Although serum prolactin levels increase in the afternoon of proestrus, they remain at the same levels in the morning as in other stages of the estrous cycle. Since all experiments in the present studies were performed in the morning, the stages of the estrous cycle were disregarded. It was noted that an increase of serum prolactin 5 min after ether stress was not observed in a group of nonhandled female rats. This might be interpreted by such as the interaction of steroids in the stress-induced release of prolactin (13).

Summary. Ether inhalation for 1 min significantly increased serum prolactin levels in both male and female rats. Serum prolactin levels reached a peak within 2.5 min after exposure to ether vapor and returned to resting levels by 15 min. Handling of female rats for 4 days lowered serum prolactin levels but the same procedure raised prolactin levels in male rats.

Injection of 3.5 mg of pentobarbital/100 g of body wt ip increased serum prolactin levels in female rats over a period of 60 min as compared with saline-injected controls; the peak was reached around 30 min after injection. In male rats, however, the same dose of pentobarbital induced only a slight increase in serum prolactin. It required injection of 5 mg pentobarbital/100 g of body wt for male rats to cause a significant increase of serum prolactin. The pattern of serum prolactin changes after 5 mg of pentobarbital in male rats was similar to that obtained in

female rats with a smaller dose. These results indicate that serum prolactin levels are readily influenced by handling, ether inhalation and pentobarbital, but the extent of these changes varies between the sexes.

Addendum. During the preparation of this manuscript, W. Wuttke and J. Meites reported the effects of ether and pentobarbital on serum prolactin and LH levels in *proestrous rats* [Proc. Soc. Exp. Biol. Med. **135**, 648 (1970)]. There was some discordance between their findings and ours, which could be explained by the difference in time and method of blood collection, duration of ether inhalation, and dose of pentobarbital.

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