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RAPID COMMUNICATION

EFFECTS OF HANDLING AND ETHER ANESTHESIA ON SERUM PROLACTIN LEVELS IN THE GOLDEN HAMSTER

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ABSTRACT: The effects of handling and ether stress on serum prolactin (PRL) levels in gonadally active and gonadally regressed male golden hamsters and in females in different stages of the estrous cycle were examined. Males, regardless of reproductive state or photoperiod, showed no significant alterations in serum PRL levels following exposure to ether and cardiac puncture, in contrast to females, which showed a significant elevation in serum PRL following these two procedures. This increase was small and inconsistent in females etherized for 1 min, but it was very pronounced in females etherized for 6 min. In females there was also a slight but significant increase in PRL following transfer from the animal room to the laboratory. Thus, routine experimental manipulations, such as moving the animals to a laboratory and ether exposure, induce significant and variable elevations of serum prolactin in female hamsters.

Early studies examining the effects of stress on pituitary hormones showed that non-specific stresses initiate lactation, presumably by increasing plasma prolactin (1). In female rats, ether anesthesia causes marked increases in plasma PRL (2,3), the magnitude of the effect being dependent on the stage of the estrous cycle (4). However, in male rats, the stress-induced increases in plasma PRL are much smaller (5,6) and difficult to detect (2).

In golden (Syrian) hamsters, the effects of stress on PRL release have not been thoroughly examined. However, several reports have indicated little, if any, effect of stress in males. Goldman *et al.* (7) reported no significant differences in serum PRL levels between males decapitated with or without prior ether exposure. Similarly, plasma PRL levels were reported not to be influenced by handling or method of blood collection (8; Goldman and Bartke, unpublished).

These previous studies examining changes in plasma levels of PRL in the hamster involved the use of a heterologous radioimmunoassay for rat PRL. This assay has since been shown to be relatively insensitive for measurement of hamster PRL (9,10). Thus, with the recent development of a sensitive homologous radioimmunoassay for hamster PRL, it became possible to re-examine the effects of stress on peripheral PRL levels in this species. Borer *et al.* (9), using a homologous radioimmunoassay, were able to detect significant effects of ether stress on plasma PRL in female hamsters. In this study, we examine the stress effects of routine experimental procedures on serum PRL levels in male and female hamsters in different reproductive conditions.

MATERIALS AND METHODS: Adult golden (Syrian) hamsters were purchased from Charles River Lakeview or raised in our animal colony in a long photoperiod, 14 h of light, 10 h of darkness (14 L:10 D; lights on at 0700 h). They

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were maintained in either 14 L:10 D or in a short photoperiod (5 L:19 D) at a constant temperature (22 ± 2°C) with constant access to food (Wayne Breeder Blox) and water. Females were maintained in 14 L:10 D. Estrus or the day of ovulation was monitored by checking the females daily for post-ovulatory vaginal discharge.

Blood for hormone analysis was collected in the morning by decapitation or by cardiac puncture following ether anesthesia. Serum was collected, stored at -20°C and shipped on dry ice from San Antonio to Santa Cruz for measurement of PRL.

Serum PRL levels were measured using a homologous radioimmunoassay (10) and are expressed in terms of purified hamster PRL. The assay is highly specific (<5% non-specific binding) and a sensitivity of 0.5 to 1.0 ng/ml.

Experiment 1. Males were maintained in either 14 L:10 D (18 animals) or 5 L:19 D (12 animals) for 9.5 weeks. Females (18 animals) were maintained in 14 L:10 D and grouped according to stage of estrous cycle. Half of the animals in each group were bled by decapitation within 1-3 minutes in an adjacent procedure room. The other animals were bled by cardiac puncture under ether anesthesia in the laboratory located some distance from the animal room.

Student's t-test was used to determine level of significance.

Experiment 2: Females were maintained in 14 L:10 D and grouped according to stage of estrous cycle. The animals were then divided into three groups as follows: One group of diestrous, metestrous, and estrous females was decapitated in a procedure room in close proximity to the animal room. A second group of diestrous, metestrous, and estrous females was moved upstairs some distance to the laboratory, and then bled by decapitation. A third group of diestrous and metestrous females was etherized and then bled by cardiac puncture either one or six minutes after initial ether exposure; they were then sacrificed by decapitation one hour following their initial exposure to ether.

Data were log-transformed and analyzed using two-way analysis of variance to test for homogeneity of the data. Student-Newman-Keuls test was used to determine the level of significance among treatment groups. Paired t-tests were done to determine the effect of bleeding order.

RESULTS: *Experiment 1.* Serum PRL concentrations in male hamsters rapidly decapitated in the procedure room and those bled by cardiac puncture under ether anesthesia in the laboratory were not significantly different (Fig. 1). There was also no effect of photoperiod on this response. Chronic exposure to 5 L:19 D produced the expected suppression of serum PRL levels (11,12). In contrast, females bled with ether anesthesia by cardiac puncture demonstrated higher serum PRL as compared to females decapitated in the procedure room. This elevation in serum PRL following stress was significant only in diestrous females ($p < 0.001$).

Experiment 2: There was a slight but significant increase in serum PRL ($p < 0.05$) in females moved to a laboratory and subsequently decapitated as compared to those decapitated rapidly after removal from the animal room (Fig. 2). There was no significant effect of stage of the reproductive cycle, estrus, diestrus or metestrus, on this response.

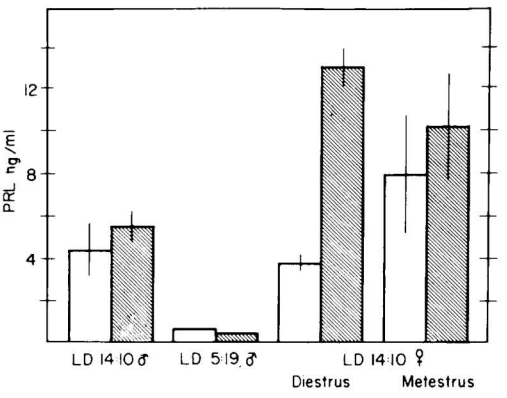

Fig. 1. Plasma prolactin level in male and female hamsters following exposure to ether and cardiac puncture ▨ or decapitation □ . Vertical lines represent the standard error about each mean. Where no standard errors are visible, they are occluded by the bar.

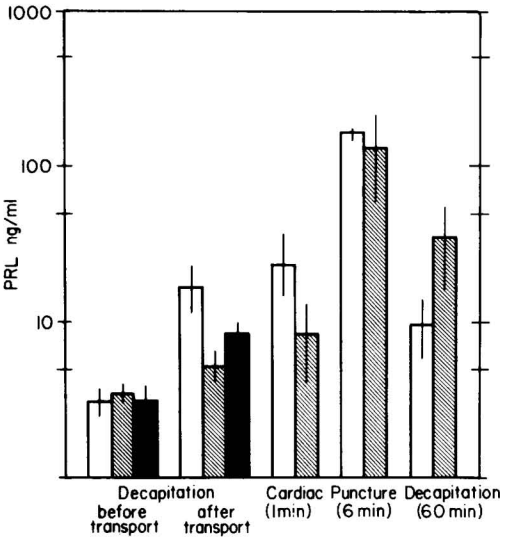

Fig. 2. Plasma prolactin levels in female hamsters in different stages of the reproductive cycle, diestrus ▭, metestrus ▨, estrus ■ following exposure to ether and cardiac puncture, or decapitation. Vertical lines represent the standard error about each mean.

Following exposure to ether for one minute, there was an apparent but not statistically significant increase in serum PRL. However, following a six-minute exposure to ether, there was a striking increase in serum PRL ($p < 0.05$). Sixty minutes following exposure to ether, PRL levels had declined to levels not significantly different from those measured in animals decapitated upstairs, or bled following a one-minute exposure to ether. There was no significant effect of order of bleeding on serum PRL.

DISCUSSION: Our findings indicate that, in male hamsters, there is no significant effect of ether stress, handling or cardiac puncture on serum PRL levels. These results are consistent with previous observations in the hamster (7; Goldman and Bartke, unpublished) but differ from results in male rats (13). However, in female hamsters there is a significant effect of ether and handling stress on serum PRL levels. This effect is most pronounced in diestrous females. These results are similar to those seen in female rats, which demonstrate a marked increase in stress-related PRL release on the morning of diestrus I and the afternoon of diestrus II. Additionally, in ovariectomized rats, stress-induced PRL release is not observed, which suggests that estrogen and/or progesterone may influence this response (4).

Since females were shown to be much more sensitive to stress than were males, they were used in the second series of experiments to separate the relative contributions of various experimental procedures to this stress-induced increase in PRL. Results indicate that there was a significant effect of moving the animals to the laboratory. Ether exposure for one minute, followed by cardiac puncture, did not significantly increase PRL above these already elevated levels. However, a six-minute exposure to ether caused a significant increase in serum PRL. This increase appeared to be greater in diestrous as compared to metestrous females, but this difference was not statistically significant. PRL levels in female hamsters return to This marked increase in serum PRL was no longer evident in animals bled 60 minutes after initial ether exposure. The decrease appeared to be greater in diestrous as compared to metestrous females, but, again, this difference was not significant. Borer *et al.* (9) also reported that plasma normal one hour following ether stress. There was no significant effect of stage of estrous cycle on the stress-induced increase in serum PRL. However, diestrous females seemed to respond to stress with an immediate increase in PRL of short duration, and metestrous females with an increase in PRL that was delayed, but of a longer duration.

There was also no significant effect of bleeding order on serum PRL. However, the lowest mean levels of PRL measured in our experiments were in animals decapitated in a room adjacent to the animal quarters and in the first animals removed from the cage.

In conclusion, in female golden hamsters, unlike males of this species, several procedures commonly involved in collection of blood samples (moving, handling, ether exposure and cardiac puncture) result in significant

elevations in serum PRL levels. The most dramatic elevation in PRL was observed in animals exposed to ether for six minutes. It is important to note that PRL responses to stress are highly variable among females, partially due to effects of the reproductive state of the animal. It should also be emphasized that essentially all routine experimental procedures resulted in significant or apparent elevations in serum PRL above the "basal" levels in presumably unstressed females sacrificed near the animal room by decapitation without ether anesthesia.

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