# Effect of Implantation of Anti-LH Serum Into Median Eminence on Rat Pituitary and Serum LH[1] (36535)

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It is a generally accepted concept that the functional axis of the hypothalamus, the anterior pituitary and the gonads is controlled by steroidal feedback actions, in which the facilitating or inhibiting information for gonadotropins comes from the peripheral target glands. It has been suggested recently that the pituitary hormones themselves might affect the hypothalamus, especially the median eminence (ME), to control the secretion of the pituitary hormones by a "short" feedback action (1). Many studies have been carried out in various kinds of experimental designs, *i.e.*: (a) electrophysiological study after exogenous pituitary hormone administration (2–4), (b) implantation of these hormones in the brain (5–10), (c) effect of hypophysectomy on hypothalamic releasing hormones (11–15), (d) histological and histochemical study of the central nervous system (14) and (e) *in vitro* culture of hypothalamic tissue with pituitary hormones (15–16). Studies concerned with this "short" feedback action of luteinizing hormone (LH) and hypothalamic control of LH secretion have been reported (5–7). Since in these studies mentioned above (5–7) a large amount of exogenous LH was implanted directly into the rat ME, it would be difficult to distinguish the physiological influence of endogenous LH on the hypothalamic ME. In the present study, the effects of an anti-LH serum (A/S) implant into the hypothalamic region on the levels of pituitary and serum LH are described.

*Materials and Methods. Animals.* Eighty-five to 95 day old virgin cycling female rats of the Sprague-Dawley strain (Holtzman Co., Madison, WI) were used. They were caged in groups of 3–4, fed Purina rat chow and water *ad libitum*, and maintained on an artificial light regimen of 14 hr light and 10 hr darkness. In Expt. 1 of A/S implant, rats were ovariectomized 7 days before A/S implant and sacrificed 2 days later and in Expt. 2 rats were ovariectomized 14 days before the implantation and killed 6 days later. The ovariectomy was done by dorsolateral incision under ether anesthesia.

*Preparation and characterization of antisera (A/S).* NIH-LH-S14 was used to produce A/S in New Zealand white female rabbits (17) and nonspecific antibodies were removed and the A/S was characterized (18). The antibody content of A/S as determined by the quantitative precipitin test was between 1 and 2 mg/ml of serum. The A/S used in this experiment showed 50% binding with 5 pg of $^{125}$I-LH (ovine) at 1:3000 dilution. The ability of this A/S to neutralize endogenous rat LH was tested by injecting an increasingly large single dose of the A/S into day 8 pregnant rats in order to ascertain the minimal dose that would induce vaginal bleeding and resorption of fetuses (18). The minimal dose of A/S that consistently terminated pregnancy was 400 μl. This A/S reacted with rat LH but showed no cross-reaction with sheep or rat prolactin by the Ouchterlony agar-gel double diffusion test (19).

*ME implantation.* A/S (7–11 μl) was mixed with equal amounts of cocoa butter and implanted stereotaxically into the ME of ovariectomized rats under ether anesthesia

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TABLE I. Effect of LH A/S Implants into Rat Median Eminence on Pituitary Content and Serum Concentration of LH.

| Expt.[a] | Group | No. rats | LH[b] Anterior pituitary (μg/mg) | LH[b] Serum (ng/ml) |
|---|---|---|---|---|
| 1 | NRS Control | 7 | 1.58 ± 0.08[c] | 11.9 ± 3.1 |
| | LH A/S | 6 | 1.12 ± 0.10[d] | 12.9 ± 2.9 |
| 2 | NRS Control | 7 | 1.78 ± 0.34 | 20.5 ± 2.7 |
| | LH A/S | 5 | 0.66 ± 0.23[e] | 18.5 ± 6.4 |

[a] See under "Materials and Methods" for description of the experimental procedure in Expts. 1 and 2.

[b] Equivalents of NIAMD Rat LH-I-1.

[c] Values are mean ± SD.

[d] $p < 0.025$.

[e] $p < 0.05$.

using a 20-gauge stainless steel needle containing this mixture. The needle was fixed directly to the skull with dental resin as described earlier (20). In control groups, the needle containing only normal rabbit serum (NRS) in cocoa butter was implanted in the same manner. In another group of rats, implantation of LH (NIH-LH-S14, approx 100–150 μg) was carried out. All animals were sacrificed by decapitation 2 or 6 days after implantation. The brain was fixed in 10% formalin and examined histologically for precise site of needle tips.

*Radioimmunoassay of LH.* Blood was collected just after decapitation, centrifuged and serum was frozen at —20° until assayed. The anterior pituitaries were removed immediately, weighed individually and homogenized in exactly 0.5 ml of 0.9% saline. After centrifugation, the supernatant was kept frozen until assayed for LH. Luteinizing hormone concentration in the sera and pituitary extracts from animals were measured by double antibody radioimmunoassay technique (21). Purified rat LH (NIAMD-Rat LH-I-1) was used as a reference preparation (biological potency = approx 1.0 × NIH-LH-S1/mg). Student's *t* test was used to determine significance of difference between control and treated groups.

*Results.* Implantation of A/S into the ME significantly decreased pituitary content of LH. The effect was more pronounced 6 days after implantation than 2 days (Table I). However, serum LH concentration was not significantly changed by implantation of A/S into ME.

In LH-treated rats, pituitary LH content was decreased to 1.13 ± 0.04 μg/mg compared to 1.58 ± 0.08 μg/mg for the control animals. The difference was significant at the .01 level (Table II).

At autopsy the anterior pituitary, uterus, and adrenal glands were removed and weighed. No significant difference in weights of these organs was observed between control and A/S treated animals.

*Discussion.* Our present data indicate that placement of anti-LH serum in the hypothalamic ME reduced the pituitary LH content in rats. Since this A/S was specific to ovine LH and able to neutralize rat LH (18), it appears that the endogenous LH in the rat may participate in the control of LH synthesis by a positive feedback action on the hypothalamohypophyseal axis. Recent investigators have demonstrated a positive feedback action of gonadotropins on this axis. FSH, if it is implanted in the hypothalamus of 31–32 day old immature rats, stimulates the secretion of endogenous FSH through a positive feedback mechanism (8). In estrogen primed ovariectomized rabbits, the EEG was influenced by the endogenous gonadotropins released by coitus (3) and systemic administration of pituitary and placental hormones was seen to alter brain activity (4). Recently, on the basis of multiple-unit activity in

TABLE II. Effect of LH Implant into Rat Median Eminence on Pituitary Content and Serum Concentration of LH.[a]

| Group | No. rats | LH[b] Anterior pituitary (μg/mg) | LH[b] Serum (ng/ml) |
|---|---|---|---|
| NRS Control | 7 | 1.58 ± 0.08[c] | 11.9 ± 3.1 |
| LH | 5 | 1.13 ± 0.04[d] | 10.1 ± 1.3 |

[a] NRS = normal rabbit serum.
[b] Equivalents of NIAMD Rat LH-I-1.
[c] Values are mean ± SD.
[d] $p < 0.01$.

the rat hypothalamus, it was suggested that LH exerted a positive feedback action on brain pituitary function (25). Our data on pituitary content of LH after A/S implantation into the median eminence can be interpreted to support this concept. A number of studies have been reported to prove the positive feedback action of other pituitary hormones (1–2, 14, 22–24).

The data obtained in this study also provide further evidence that exogenous LH implanted into the ME will significantly reduce pituitary LH content. This result is in agreement with the observation of other investigators (5–7); however, they found decreased levels of serum LH in their experiments.

It is also important to consider whether endogenous LH is normally present in the hypothalamic ME. Several studies in which luteinizing hormone activities were found in hypothalamic extracts have supported this hypothesis (26–28). Furthermore, anatomical evidence of a vascular system from the posterior surface of the anterior pituitary into the capillary complex of the median eminence has been demonstrated in histological studies (29–30). This anatomical connection may be a possible route, through which controlling signals may reach the ME directly from the pituitary.

The pituitary hormones, GH, ACTH, and prolactin have been reported to reduce the pituitary weight in rats. On the other hand, LH has been observed to have no significant effect on anterior pituitary weight (5–7). Our data are in good agreement with these studies. A/S implants also did not have any significant effect on pituitary weight. Body weight, adrenal, and uterine weights were also unchanged.

*Summary.* A specific rabbit anti-sheep LH serum (A/S) was implanted into the rat hypothalamic median eminence (ME) to investigate the mode of action of a "short" feedback of endogenous LH by neutralization of this hormone at its presumed site of action in the brain. Rats were ovariectomized 7 or 14 days before A/S implantation and sacrificed 2 or 6 days later. The pituitary and serum LH levels were measured by a radioimmunoassay technique. Implantation of A/S resulted in a significant decrease in pituitary content of LH but no change in concentration of serum LH. Implantation of sheep LH in ME of ovariectomized rats also reduced pituitary LH content significantly with no effect on serum LH. LH A/S implantation did not alter gross weight of the anterior pituitary or the target organs of its tropic hormones. These data suggests that while an unphysiologically large amount of exogenous LH implanted directly into ME exhibits a negative effect on pituitary content of LH, endogenous LH appears to exert a positive short feedback action on the hypothalamo-pituitary axis.

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