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Increased *in vitro* Pituitary Response to LH-RH After *in vivo* Estrogen Treatment[1] (38128)

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Treatment of ovariectomized heifers with estradiol-17B (E_2) has been shown to elicit a biphasic LH response indicative of the positive and negative effects of E_2 on LH release (1). Thus, the initial effect of E_2 on LH secretion was one of depression lasting 3–10 hr. This was followed by a marked rise in plasma LH levels some 12–20 hr after treatment. Similar patterns have been described for sheep (2), monkeys (3) and humans (4).

In an attempt to determine whether E_2 was acting at the hypothalamic level, the pituitary, or both sites to elicit the biphasic response, 4 parameters associated with LH release were measured at times of maximal LH depression and elevation after E_2 treatment. These parameters included: (a) pituitary LH content, (b) hypothalamic LH-RH content, (c) plasma LH levels, and (d) the *in vitro* response of the pituitary to a standardized dose of hypothalamic extract (HE). Additional experiments were included to study the *in vitro* effects of estrogen and potassium on pituitary LH release from superfused bovine pituitary tissue.

Materials and Methods. Animals. Fifteen Holstein heifers, ovariectomized at least 30 days in advance, were assigned to 3 treatment groups of 5 animals each. Animals in the control group were slaughtered without treatment, while those in the 2 remaining groups were slaughtered either 3 or 18 hr after injection of E_2 (2 mg sc). The times were selected to correspond, respectively, to the times of maximum depression and elevation of peripheral LH levels after E_2 injection (1).

Sample Collection. Blood samples for plasma LH analyses were collected from the jugular vein 3 hr prior to estrogen injection and at 3 hr intervals thereafter until slaughter. Immediately after slaughter, pituitaries and hypothalami were excised and placed in physiologic saline (4°). Landmarks for removal of the hypothalami were those described by Wilks and Hansel (5). Hypothalami were cleaned, weighed and frozen for later analyses of LH-RH content. Anterior pituitaries were dissected free from surrounding connective tissue and the posterior lobes discarded. The anterior portion was finely minced, washed three times in physiologic saline and aliquots (70–100 mg) were placed in superfusion chambers.

Superfusion. The superfusion procedure was essentially that described by Zolman and Convey (6). Medium (Krebs–Ringer bicarbonate with glucose [2 mg/ml]) was pumped from a gas-tight reservoir through the superfusion chambers at a rate of 0.20 ml/min/chamber. Chambers and reservoirs were maintained at 37° in a water bath. Silicone tubing was used throughout the system. Superfusion chambers were made from constricted portions of Pasteur pipets and plugged with glass wool to prevent escape of tissue. After superfusion for 30 min, the tissue response to HE was measured with the addition of HE into each channel by means of a three-way valve arrangement. Immediately before addition, the HE was adjusted to pH 7.4 with

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2 *N* NaOH and diluted with the medium. Extracts of cerebral cortex, treated in the same way, served as controls.

Preparation of hypothalamic extract. Bovine hypothalami taken from animals at a slaughter house were pooled and extracted by homogenization in cold 0.1 *N* HCl (5 ml/hypothalamus). The supernatant was collected after centrifugation (12,000 *g* for 1 hr) and was subjected to ultrafiltration to remove components over 10,000 mol wt (Amicon Corporation, UM-10 membrane, 30 psi). The purpose of this step was two-fold; to remove all immunoreactive LH, and to reduce the protein content of the extract. The filtered solution was collected, freeze-dried, reconstituted to a concentration of 4 hypothalami/ml of saline, and frozen. Hypothalami from experimental animals were individually prepared in the same manner, except that the ultrafiltration step was omitted, because of insufficient sample volume.

LH Radioimmunoassay. A previously described (7) solid phase radioimmunoassay was employed for all LH determinations. Effluent samples collected from the superfusion were diluted in assay buffer (1:200) and 20-μl portions were assayed in duplicate. Pituitaries were homogenized in the same buffer and assayed in triplicate at two concentrations. Rat plasma was assayed in triplicate using a modification of the bovine LH system as described by Gombe *et al.* (8). Results of both assays are reported in terms of bovine LH (NIH-LH-B7). *LH-RH Assay.* A bioassay for LH-RH, based on the systems described by Ramirez and Mc Cann (9) and Gay *et al.* (10), was used to estimate the LH-RH content of bovine hypothalami. Ovariectomized rats (150–200 *g*) were primed with an injection of 5 mg progesterone and 50 μg estradiol benzoate 48 hr before the assay. HE equivalent to 0.1 hypothalamus was injected into the jugular vein. Blood samples were collected immedately before and 5 min after the injection. The increase in circulating LH was used as an index for comparison of LH-RH content of the bovine hypothalami. Hypothalamic extracts were assayed for their LH content to ascertain that the increase of LH in rat plasma was not due to bovine LH contained in the HE. A further control was provided by assessing the ability of an extract of bovine cerebral cortex to elevate the LH concentration of rat plasma.

Results. Administration of E_2 to ovariectomized heifers resulted in the expected biphasic pattern of plasma LH levels (1). LH levels were depressed at 3 hr and elevated 18 hr after treatment (Fig. 1). The increased plasma LH levels observed 18 hr after treatment were associated with a significant depression in the pituitary LH contents at this time (Table I). However, depressions of plasma LH levels observed at 3 hr were not associated with any apparent change in pituitary LH content (Table I).

Measurements of hypothalamic LH-RH content revealed a depression in the group measured 18 hr after treatment. However, the depression was not statistically significant because of variation between animals within this group. This variation does not appear to be inherent in the bioassay system for LH-RH,

TABLE I. The Effect of *in vivo* Estradiol Treatment on Pituitary LH Content and Hypothalamic LH-RH Content.

Treatment group (hr after estradiol treatment)	Pituitary LH (ng/mg wet weight)	Hypothalamic LH-RH (ng/mg)[a]
Control	3955 ± 509[b]	3.99 ± 0.95
3	3450 ± 509	4.42 ± 0.95
18	2011 ± 509[d]	2.74 ± 0.95
Extract of bovine cerebral cortex[c]		−1.84 ± 1.27

[a] Expressed as the increase produced in peripheral plasma LH of ovariectomized rats by injection of 1/10 of the total extract of a bovine hypothalamus per rat.

[b] Mean ± S.E.

[c] Control to assure specificity of rat response to hypothalamic extract.

[d] Significantly ($P < 0.05$) lower than control or 3 hr groups.

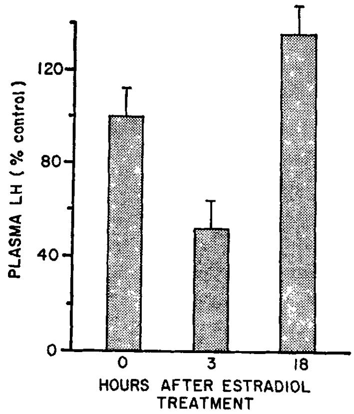

FIG. 1. Plasma LH after injection of estradiol—17β (2 mg) into heifers. Samples taken at the time of slaughter. Five animals per group, "I" bars represent S. E. Values are expressed as percent of control.

since the response of different rats to the same hypothalamic extract (HE) was sufficiently uniform to demonstrate significant differences between heifers within treatment groups.

Experiments conducted to investigate the utility of the superfusion system for measurement of pituitary response to HE indicated some between channel variation, but pituitary tissue responded to HE and did not respond to infusion of extract from cerebral cortical tissue (Fig. 2). Additional experiments demonstrated that the system released LH in response to increased levels of potassium, but did not respond to inclusion of E_2 in the superfusion medium (Table II).

Pituitary tissue from each treatment animal was superfused for 30 min, after which it was stimulated by a 10 min addition of HE to the medium (equivalent to ¼ hypothalamus per channel). The response to HE,

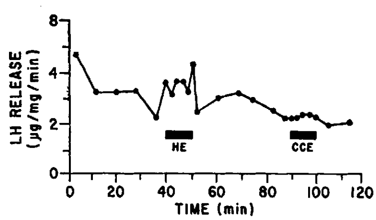

FIG. 2. The effect of addition of hypothalamic extract (HE) equivalent to 1/4 hypothalamus or cerebral cortex extract to the superfusion medium on the rate of LH release by minced bovine pituitary tissue.

measured as the increase in the amount of LH released/min/mg tissue, was significantly greater in the 18-hr E_2-treated group than in either the control or 3 hr treated group (Fig. 3). It is interesting to note, however, that the response to HE by the 3 hr group was not different from the control group at a time when peripheral levels of LH were depressed. Furthermore, addition of E_2 *in vitro* (Fig. 3) had no effect on the ability of the superfused pituitary tissue to respond to HE.

Discussion. The present observation that the *in vitro* pituitary response to HE is enhanced by estrogen injected 18 hr previously agrees with reports that etstrogen priming increases the *in vivo* LH response to LH-RH in sheep (11), and rats (12). However, the previous studies were not able to distinguish between an effect of estrogen at the hypothalamic or the pituitary level. Our data strongly suggest that this effect of estrogen represents a heightened sensitivity of the pituitary tissue to respond to a given amount of LH-RH. Nett *et al.* (13) recently reported a lack of correlation between plasma LH-RH and LH levels following estrogen administration to anestrous ewes. Their results are consistent with the idea that estrogen increases sensitivity of the pituitary to endogenous releasing hormone.

Other investigators found either no effect or suppression of the LH response to LH-RH by estrogen pretreatment of women (14, 15). The interval between estrogen injection and LH-RH stimulation in these latter reports was 4–6 hr, which corresponds to the inhibitory portion of the biphasic response observed in the present experiment. Thus, these data are consistent with the hypothesis that the stimulatory action of estrogen on LH release is, at least partly, exerted on the pituitary, and that this action is not manifest until several hours after exposure to estrogen. Since the pituitary LH content and the pituitary response to HE were not reduced in heifers slaughtered 3 hr after E_2 treatment, the reduction in blood levels of LH would appear not to represent a shift from secretion of LH to storage by the pituitary, nor a decreased sensitivity to LH-RH at the pituitary level. Instead, estrogen may act at higher centers to reduce the amount of LH-RH reaching the

TABLE II. The Effect of Inclusion of Potassium (60 m*M*) or Estradiol-17B (5 ug/ml) in the Superfusion Medium on LH Release by Minced Bovine Pituitary Tissue.

Group	Total Release[a] (ng/mg)
Control	81.3 ± 9.3[b]
Estrogen	78.3 ± 9.3
Potassium	107.7 ± 9.3[c]

[a] During a 30 min superfusion.
[b] Mean ± S.E. ($N = 24$).
[c] Significantly greater than control or estrogen ($P < 0.05$).

pituitary during the inhibitory phase, although this was not reflected by a change in hypothalamic LH-RH content.

Pituitary tissue from animals which have received E_2 18 hr previously was more responsive to HE than tissue taken from the control or 3 hr groups. This alteration, in combination with release of stored LH from the pituitary (as shown by reduced pituitary content at 18 hr), can largely account for the increase in peripheral LH levels. Since a significant decrease in hypothalamic stores of LH-RH was not observed in this experiment it cannot be stated that an additional stimulus was provided by increased amounts of LH-RH reaching the pituitary during the time of LH peak. It should be emphasized that the increased sensitivity of the pituitary

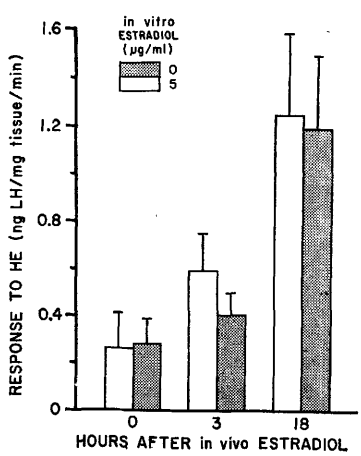

FIG. 3. The effects of *in vivo* and *in vitro* estrogen treatment on the response to hypothalmic extract by superfused bovine pituitary tissue.

tissue to HE 18 hr after in vivo E_2 treatment was demonstrated in a superfusion system in which it was not possible to demonstrate a direct *in vitro* effect of E_2 (Table II and Fig. 3). Other workers (Schneider and Mc Cann (16), Piacsek and Meites (17) and Schally *et al.* (18) have demonstrated LH release *in vitro* in response to added E_2 in incubations of rat pituitary tissue for periods of 1–6 hr. However, in agreement wth our results, none of these workers demonstrated any augmentation by E_2 of the LH-RH response *in vitro.*

Schneider and McCann noted that E_2 blocked *in vitro* stimulation of LH-RH release from stalk median eminence tissue by dopamine (16). This observation is in agreement with our suggestion that the negative effects of estrogen are exerted at the hypothalamic or higher levels, and is in agreement with the conclusion of Davidson (17) who demonstrated a negative feedback by estrogen on the medial basal hypothalamus of rats. Thus, this report supports the concept that the estrogen control of LH release has both positive and negative components which act at the pituitary and hypothalamic levels, respectively. Moreover, it suggests that these components are not only separated by site of action, but also by their temporal relationship to estrogen stimulation.

Summary. Peripheral LH levels are initially depressed and then elevated in ovariectomized heifers after injections of estrogen. The sites of the estrogen induction of this biphasic response were investigated. Plasma and pituitary LH levels, hypothalamic LH-RH levels and the *in vitro* response of pituitary tissue to hypothalamic extract (HE) were measured 3 and 18 hr after estrogen injection into ovariectomized heifers. LH levels were significantly depressed 3 hr after estrogen treatment. However, none of the other parameters were different from corresponding control group data at this time. Plasma LH was elevated, pituitary LH was depressed and the *in vitro* pituitary response to HE was significantly enhanced 18 hr after estrogen treatment. These data suggest that the initial, suppressive effect of estrogen is exerted at the hypothalamic or higher levels and that the LH-releasing effect of estrogen is at the pituitary level. The positive feedback effect of estrogen on the pitui-

tary is exerted only after several hr exposure to estrogen and acts on the pituitary both by enhancing its response to LH-RH and by releasing stored LH.

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1. Hobson, W. C. and Hansel, W., Biol. of Repro. **7**, 136 (1972).

2. Goding, J. R., Blockey, M. A. deB., Brown, J. M., Catt, K. J., and Cumming, I. A., J. Repro. Fert. **21**, 368 (1970).

3. Yamaji, T., Dierschke, D. J., Hotchkiss, J., Bhattacharya, A. N., Surve, A. H. and Knobil, E., Endocrinology **89**, 1034 (1971).

4. Yen, S. S. C., and Tsai, C. C., J. Clin. Endocrinol. Metab. **33**, 882 (1971).

5. Wilks, J. W. and Hansel, W., J. Animal Sci. **33**, 1042 (1971).

6. Zolman, J., and Convey, E. M., Proc. Soc. Exp. Biol. Med. **140**, 194 (1972).

7. Hobson, W. and Hansel, W., Endocrinology **91**, 185 (1972).

8. Gombe, S., Apgar, J. and Hansel, W., Biol. Repro. **9**, 415 (1973).

9. Ramirez, V. D. and McCann, S. M., Endocrinology **73**, 193 (1963).

10. Gay, V. L., Niswender, G. D., and Midgley, A. R. Jr., Endocrinology **86**, 1305 (1970).

11. Reeves, J. J., Arimura, A., and Schally, A. V., Biol. Repro. **4**, 88 (1971).

12. Arimura, A. and Schally, A. V., Proc. Soc. Exp. Biol. Med. **136**, 290 (1971).

13. Nett, T. M., Akbar, A. M., and Niswender, G. D. J., Animal Sci. **37**, 322 (1973) (Abst).

14. Thompson, I. E., Arfania, J. and Taymor, M. L., J. Clin. Endocrinol. Metab. **37**, 152 (1973).

15. Siler, T. M., Vandenberg, G., Rebar, R. W., Ehara, Y. E., Yen, S. S. C., Biol. Reprod. **9**, 108 (1973).

16. Schneider, H. P. G., and McCann, S. M., Endocrinology **87**, 330 (1970).

17. Piacsek, B. E. and Meites, J., Endocrinology **79**, 432 (1966).

18. Schally, A. V., Redding, T. W. and Arimura, A., Endocrinology **93**, 893 (1973).

19. Davidson, J. M. In (Ganong, W. F. and Martini, L. eds.), Frontiers of Neuroendocrinology, Oxford Univ. Press, New York (1969).

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