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Activation of the Hypothalamo-Anterior Pituitary Corticotropin-Releasing Hormone, Adrenocorticotropin Hormone and β-Endorphin Systems During the Estradiol 17β-Induced Plasma LH Surge in the Ovariectomized Monkey

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The present work describes time-dependent changes in the content of corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH), and β-endorphin (β-EP) in the hypothalamus (HT) and anterior pituitary (AP) and in the concentration of ACTH and β-EP in the plasma during the 17β estradiol (E_2) benzoate (E_2B)-induced luteinizing hormone (LH) surge in ovariectomized cynomolgus monkeys. Monkeys were euthanized at 0, 30, 48, 72, and 96 hr post-E_2B. HT and AP were rapidly dissected, extracted in 2 N acetic acid containing 1 mM phenylmethane sulfonyl fluoride at 4°C, and centrifuged at 18,000*g* for 30 min. Peptide concentrations were measured in the supernatant by specific radioimmunoassays (RIAs). In the HT, there were significant ($P < 0.05$) decreases in ACTH and β-EP content by 30 hr post-E_2B and a significant ($P < 0.05$) decrease in HT CRH content 48 hr post-E_2B. Thereafter, CRH, ACTH, and β-EP content increased up to 72 hr post-E_2B. In the AP, there was an almost linear decrease in the CRH content through 48 hr post-E_2B followed by a marked 20-fold ($P < 0.01$) increase in the AP CRH content at 72 hr post-E_2B, which corresponds to the time of the descending arm of the LH surge. The patterns of ACTH and β-EP content were very similar in the AP, while that of CRH differed markedly. In contrast, in the HT CRH, ACTH, and β-EP profiles were very similar. Significant ($P < 0.05$) increases in circulating levels of ACTH, β-EP, and cortisol were evident at 30 hr (all 3 hormones), 48 hr (β-EP and cortisol), and 72 hr (cortisol) post-E_2B, which corresponds with the time of decreased hypothalamic content of CRH, ACTH, and β-EP. These results suggest that there maybe a marked activation of the hypothalamo-anterior pituitary-adrenal axis during the negative and positive feedback phases of the E_2B-induced LH surge in the ovariectomized monkey.

Key words: hypothalamus, anterior pituitary, CRH, ACTH, β-endorphin

INTRODUCTION

The ovulatory midcycle luteinizing hormone (LH) surge in primates is the culmination of many complex interactions between the hypothalamo-pituitary-ovarian axes. Although it is generally acknowledged that the primary interplay is between ovarian estradiol and hypothalamic GnRH secretion, there are a myriad of additional neuroendocrine factors whose contributions remain incompletely understood. Among these factors, it is becoming increasingly recognized that there is a complex interaction between the adrenocorticotropic axis and the gonadotropic axis (Mahesh and Brann, 1992). In nor-

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mally cycling primates and rodents, an increase in the activity of the corticotropic axis has been reported around the time of ovulation. Serum levels of adrenocorticotropin (ACTH) and corticosterone are elevated prior to the start of the preovulatory LH surge on the afternoon of proestrus in the rat (Raps et al., 1971; Buckingham et al., 1978; Brann and Mahesh, 1991) and a midcycle elevation of ACTH and cortisol has been reported in women (Genazzani et al., 1975). Furthermore, it has been shown that two peptides co-localized in the gonadotrope cells of the anterior pituitary (Leu-Enkephalin with LH cells and ACTH with follicle-stimulating hormone [FSH] cells) can stimulate the release of gonadotropins in primary culture of anterior pituitary cells (Slama et al., 1990). Here, we hypothesize that 17β estradiol (E_2) secreted from the dominant follicle may concomitantly activate the corticotropic and gonadotropic axis.

In the ovariectomized (OVX) monkey, a "midcycle-like" LH surge can be initiated by administration of E_2 benzoate (E_2B) (Karsch et al., 1973; Nakai et al., 1978; Knobil, 1980; Knobil and Hotchkiss, 1988). Additionally, it has also been shown that progesterone and 17α-hydroxyprogesterone will advance this induced LH surge (Schenken et al., 1985a,b). We have recently reported that in vivo E_2B exposure of OVX monkeys leads to a sequential increase in the secretion of β-endorphin (β-EP), gonadotropin-releasing hormone (GnRH), and substance P (SP) from hypothalamic tissue in vitro (Kerdelhué et al., 1992). These results suggest to us that an activation of the POMC system precedes the activation of the GnRH secretory system.

The effects of E_2 on pro-GnRH-GAP and its products in the primate hypothalamus (HT) and anterior pituitary (AP) of OVX monkeys have also been reported (Kerdelhué et al., 1993). Changes in GnRH and GnRH-associated peptide (GAP) at both hypothalamic and AP levels were closely related at all times after E_2B treatment. However, the pattern of change in the AP was very different from that in the HT. In the HT, pro-GnRH-GAP levels did not change significantly throughout the experimental period. In the AP, the pro-GnRH-GAP increased 48 hr post-E_2B treatment, concomitant with elevated serum LH levels at the start of the LH surge. An 8-fold increase in AP GnRH occurred 30 hr post-E_2B treatment. The present in vivo study was designed to evaluate the dynamics of corticotropic hypothalami-anterior pituitary events occuring in response to an E_2B bolus in OVX monkeys. Here, we describe the changes in HT and AP corticotropin-releasing hormone (CRH), ACTH, and β-EP contents and plasma concentrations of ACTH, β-EP, and cortisol during an E_2B-induced LH surge in the OVX monkey.

MATERIAL AND METHODS

Cynomolgus monkeys (*Macaca fascicularis*) were housed in individual cages under environmentally controlled conditions (light, 12 hr; dark, 12 hr; temperature 23–25°C) and fed a diet of commercial monkey chow (Agway, Elizabeth City, NJ). Water was available ad libitum.

Hypothalamic and Anterior Pituitary Content of CRH, ACTH, and β-Endorphin

Thirty-eight long-term OVX adult female cynomolgus monkeys (*Macaca fascicularis*) weighing 2.5 to 3.5 kg were used for this study. The treatments used herein were reported in detail previously (Kerdelhué et al., 1993). Briefly, primates were used in three independent experimental series. In the first, 12 OVX monkeys were randomly divided into four equal groups. At 0 hr, three OVX monkeys were assigned as controls and received no further treatment, while the nine other OVX monkeys received a subcutaneous (sc) injection of E_2B (50 μg/kg) in sesame oil at time 0 and were evenly divided between study groups of 30, 48, and 72 hr. Monkeys were anesthetized with ketamine (10 mg/kg, im) and then euthanized with an injectable nonbarbiturate, non-narcotic euthanasia solution (T-61; American Hoecht Corp, Sommerville, NJ) at the designated times of 0 (control group), 30, 48, and 72 hr relative to the injection of E_2B ($n = 3$ per time point). Blood samples (3 ml) were collected twice daily via femoral vein puncture, under ketamine-induced anesthesia (10 mg/kg, im), from time 0 until the time of euthanasia. Serum samples were obtained after centrifugation and were stored frozen at −20°C until assayed for LH and E_2, using previously validated methodologies (Goodman et al., 1977). For the second experimental series, 15 OVX monkeys were treated as described for the first series and euthanized at times 0 (control group), 30, 48, 72, and 96 hr post-E_2B ($n = 3$ per time point). For the third experimental series, 11 OVX monkeys were treated as described above and euthanized at times 0 (control group; $n = 2$), 30 ($n = 2$), 48 ($n = 2$), 72 ($n = 2$), and 96 ($n = 3$) hr post-E_2B. Thus, the total number of monkeys studied at each time point were 8 for times 0 (control group), 30, 48, and 72 hr and 6 for 96 hr post-E_2B. Time 0 was always 09:00. Immediately following euthanasia, APs were removed, and HTs were rapidly dissected. The HTs were trimmed to encompass an area bounded anteriorly by the anterior boundary of the optic chiasma, posteriorly by the posterior margins of the mammillary bodies, laterally by the lateral hypothalamic sulci, and to a depth of approximately 5 mm. The trimmed hypothalami also encompassed the upper portions of the pituitary stalk. The net weights of the AP and the

dissected HT were 20 ± 2 mg and 156.7 ± 10.9 mg, respectively.

The HT and AP from each monkey were extracted in 2 N acetic acid containing 1 mM phenyl methane sulfonyl fluoride at 4°C and the homogenates were centrifuged at 18,000*g* for 30 min. The supernatants and the pellets were separated and stored at −80°C until the determination of CRH, ACTH, and β-EP contents in supernatant. CRH, ACTH, and β-EP determinations were performed by specific radioimmunoassays (RIAs). We have previously reported results for GnRH, pro-GnRH GAP, GAP, and SP content from these same tissue samples (Kerdelhué et al., 1993).

Synthetic human CRH (Neosystem, Strasbourg, France) was used as standard and Tyr^0 CRH (Neosystem, Strasbourg, France) as tracer. The rabbit CRH antiserum was a generous gift from Dr. W. Vale (Vale et al., 1983). The limit of detection of the assay was 5 pg/tube. The intra- and interassay variation coefficients were of 6 and 7%, respectively.

The rabbit ACTH antiserum was a generous gift from Dr. Oliver (INSERM, Marseille, France). This antiserum does not cross-react with β-EP, CLIP, and ACTH 23–29, but does cross-react with ACTH 7–38 to an extent of 30% (Mélik et al., 1993). The limit of detection of the ACTH assay was 15 pg/tube. The intra-and interassay coefficient of variation were 8.2% and 9%, respectively.

β-EP was measured according to a previously described method (Kerdelhué et al., 1982). The limit of detection of the assay was 8 pg/tube. The intra- and interassay coefficients of variations were 8% and 10%, respectively.

Plasma Levels of ACTH, β-Endorphin, and Cortisol

Seven long-term OVX adult female cynomolgus monkeys (*Macaca fascicularis*) weighing 2.5 to 3.5 kgs were used for this study. Primates were each used in four experimental series.

In the first series, the seven OVX monkeys received a subcutaneous (sc) injection of E_2B (50 mg/kg) in sesame oil. Blood samples (15 ml) were drawn from jugular cannulae (inserted 1 week previously) at 0, 30, 48, 72, and 96 hr after treatment. In the second series, the same seven OVX monkeys were treated exactly as for the first series after one week of recovery. In the third and fourth series, the same seven OVX monkeys were treated 2 months later with the vehicle used for E_2B administration (sesame oil) exactly as for the first and second series. Thus, the total number of evaluations made at each time point was 14 for both the treated and the control group. Plasma was harvested by centrifugation and the red blood cells resuspended with heparinized saline and reinfused to minimize the chances of anemia developing.

An aliquot (1 ml) of the plasma sample was removed for subsequent RIA determination of LH, E_2, and cortisol levels. The remainder (6 ml) of the plasma sample was used for ACTH and β-EP quantitation by RIA following Sep Pak extractions (see below). Both aliquots were stored frozen at −20°C until assayed. LH and E_2 were measured by previously described methodologies (Goodman et al., 1977). Cortisol was determined by a commercial kit (ICN Biomedicals, Inc., Costa Mesa, CA); the sensitivity was 1 μg/dl and the intra- and interassay coefficients of variation were 12% and 17%, respectively.

Sample Preparation for RIA of ACTH and β-EP in Plasma

Sep-Pak columns (Waters Associates, Milford, MA) filled with a non-polar stationary phase (Sep-Pak C18) were used for the preparation of samples for RIA of ACTH and β-EP. Briefly, the cartridges were prepared by successive washing with 5 ml ethanol, 5 ml 8 M urea, and 10 ml H_2O before the plasma samples were introduced to the column. The columns were then washed with 10 ml H_2O and 10 ml 4% acetic acid. The peptides which were retained were eluted with 5 ml of a mixture of ethanol (90%) and acetic acid (4%). After overnight roto-evaporation of the eluted solution in a Savant Speed Vac Concentrator System (Savant Instruments, Inc., Farmingdale, NY), the residuum was reconstituted in RIA buffer.

Statistical Analysis

For each tissue source and each hormone measured, comparisons were made between the groups of animals for the five time periods relative to E_2B administration. One way or two way analysis of variance for time and treatment were performed. Duncan's test was used to assess statistically significant differences between means. A P value of <0.05 was used to define statistical significance. Values which were greater than 3 times the standard deviation (assessed via box plots) were excluded from the analysis (no more than one result per group).

RESULTS

Hypothalamic and Anterior Pituitary Content of CRH, ACTH, and β-Endorphin

The composite pattern of serum E_2 and LH concentrations have been previously published (Kerdelhué et al., 1993). They have been included as inserts in Figures 1 and 2 to aid in data interpretation. Serum LH concentrations were suppressed from 363 ± 40 to 104 ± 11

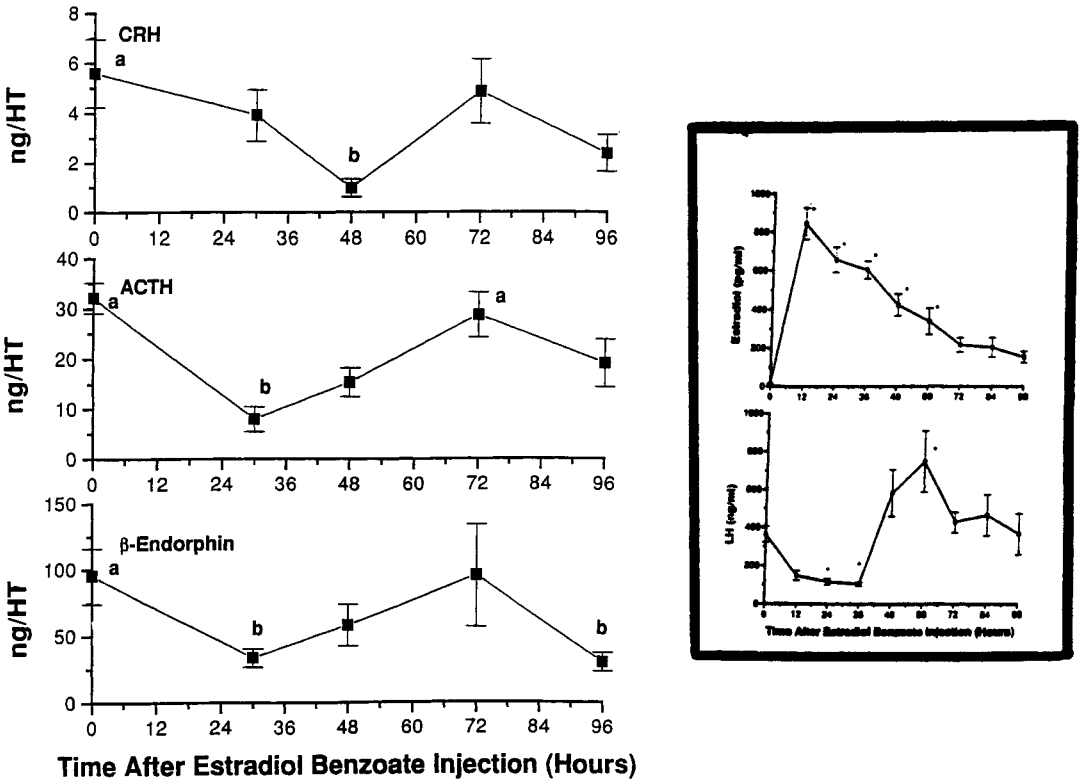

Fig. 1. Pattern of mean (± SEM) hypothalamic content of CRH, ACTH, and β-endorphin in ovariectomized cynomolgus monkeys treated with one subcutaneous injection of estradiol benzoate (50 μg/kg) at time 0 hr. For each peptide and tissue source significant differences ($P < 0.05$) between values are denoted by different letters. The boxed **insert** presents the composite pattern of mean (± SEM) serum concentrations of E_2 and LH in all blood samples taken up to the time of euthanasia from ovariectomized cynomolgus monkeys treated with one injection of estradiol benzoate (50 μg/kg) at time 0 hr. (*) denotes a significant difference ($P < 0.05$) from the values observed on time 0 hr. This figure was previously published in our earlier publication (Kerdelhué et al., Endocrinology 132: 1151–1157, 1993, Figure 1,© The Endocrine Society). It is represented here to aid in data interpretation.

ng/ml by 36 hr post-E_2B, after which the positive feedback effects of E_2 became evident. LH concentrations rose to above pre-treatment levels by 48 hr, with maximum levels (746 ± 78 ng/ml) occurring at 60 hr post-E_2B.

At the HT level (Fig. 1) there was a significant decrease in CRH content 48 hr post-E_2β and there were significant decreases in both ACTH and β-EP content at 30 hr post-E_2B. At the AP level (Fig. 2) there was a statistically significant decrease in CRH content by 48 hr post-E_2B, followed by a marked increase at 72 hr post-E_2B, returning to initial values by 96 hr post-E_2B. There were no statistically significant changes in ACTH and β-EP contents.

Plasma Levels of ACTH, β-EP, and Cortisol

Serum E_2 and LH concentrations in blood samples taken exhibited the expected values after E_2B administration (Fig. 3). During the control intervals, there were no major fluctuations in any of the hormones measured.

There were significant increases in plasma ACTH, β-EP, and cortisol concentrations relative to control at 30 hr post-E_2B (Fig. 4). The increase in plasma ACTH (Fig. 4, top) was only significant at 30 hr post-E_2B. However, the increase in plasma β-EP (Fig. 4, middle) was significant at 30, 48, and 72 hr post-E_2B and the increase in plasma cortisol (Fig. 4, lower part) was significant at 30 hr and 48 hr post-E_2B.

DISCUSSION

In vivo exposure of OVX monkeys to E_2B resulted in the expected generation of a "midcycle-like" serum LH surge. The present study documents significant variations in hypothalamic CRH, ACTH, and β-EP content, anterior pituitary CRH content, as well as plasma

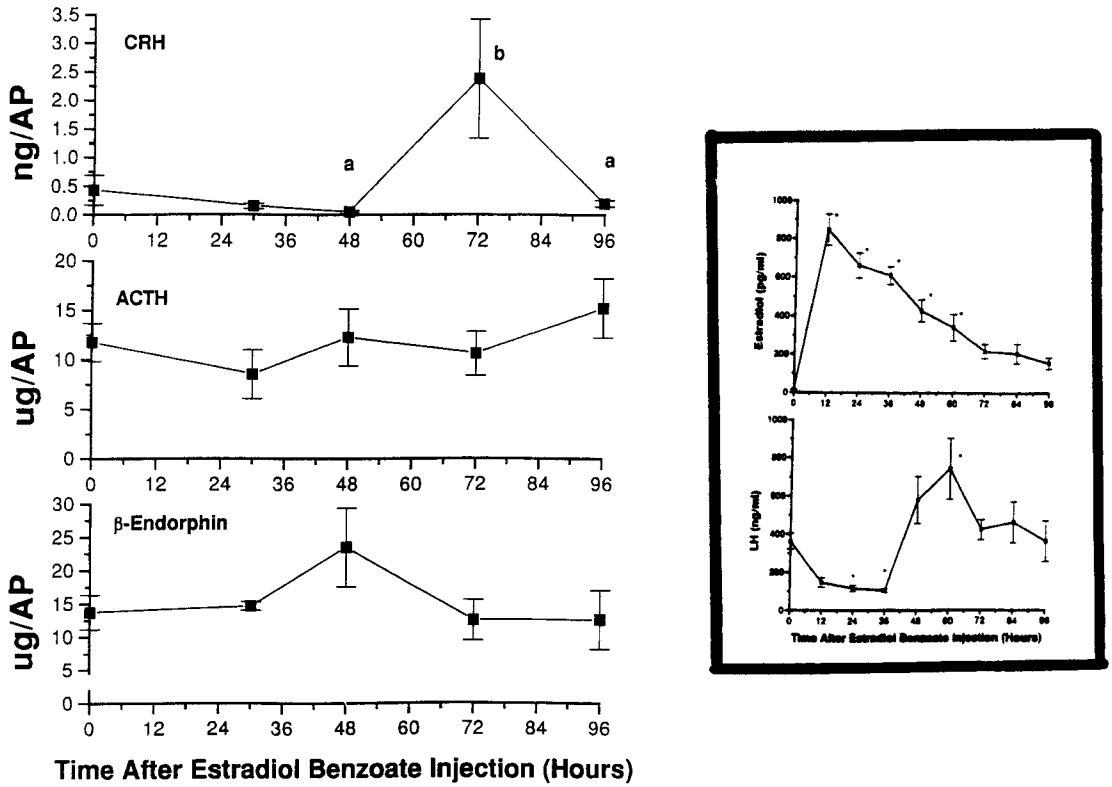

Fig. 2. Pattern of mean (± SEM) anterior pituitary content of CRH, ACTH, and β-endorphin in ovariectomized cynomolgus monkeys treated with one subcutaneous injection of estradiol benzoate (50 μg/kg) at time 0 hr. For each peptide and tissue source significant differences ($P < 0.05$) between values are denoted by different letters. The boxed **insert** presents the composite pattern of mean (± SEM) serum concentrations of E_2 and LH in all blood samples taken up to the time of euthanasia from ovariectomized cynomolgus monkeys treated with one injection of estradiol benzoate (50 μg/kg) at time 0 hr. (*) denotes a significant difference ($P < 0.05$) from the values observed on time 0 hr. This figure was previously published in our earlier publication (Kerdelhué et al., Endocrinology 132: 1151–1157, 1993, Figure 1,© The Endocrine Society). It is represented here to aid in data interpretation.

ACTH, β-EP, and cortisol concentrations during the various phases of the E_2B-induced LH surge in OVX cynomolgus monkeys. Changes in the in vitro secretion of GnRH, SP, and β-EP (Kerdelhué et al., 1992) as well as changes in the in vivo variations in HT and AP content of GnRH precursor (Kerdelhué et al., 1993) have previously been documented during the various phases of the E_2B-induced LH surge in cynomolgus monkeys. Values for maximal release of peptides in vitro occurred at different intervals: 24 and 30 hr post-E_2B for β-EP and GnRH, respectively. The findings for β-EP content in both HT and AP paralleled those of ACTH. Although the results indicate that the content of ACTH and β-EP in the AP are relatively stable after E_2 stimulation, the marked increase in serum ACTH, β-EP, and cortisol clearly shows that there was either an increase in the AP secretion of ACTH and β-EP or a decrease in clearance of these hormones. While it is possible that the elevation in plasma cortisol levels could result from estrogen-induced elevations of cortisol binding globulin we think this is unlikely since that would not explain the parallel changes in ACTH and β-EP. A similar phenomenon does not seem to occur at the HT level, where there is a marked depletion of ACTH and β-EP followed by an increase, which occurs in a reciprocal pattern to the serum concentration of ACTH and β-EP. This diminution of β-EP at 30 hr is in keeping with the known inhibitory action of β-EP on GnRH release and synthesis (Ferin et al., 1984), as well as of the occurrence of an increased GnRH release at 30 hr post-E_2B (Kerdelhué et al., 1992).

The stimulation of β-EP release (reflected in increased serum concentration) during the estradiol negative feedback phase (30 hr) is consistent with the highest levels of β-EP in portal blood being obtained from ovariectomized animals administered supplemental E_2 and progesterone and from collections performed in late follicular and mid-luteal phase on cycling monkeys (Ferin et al., 1984). Furthermore, intravenous or intracerebral

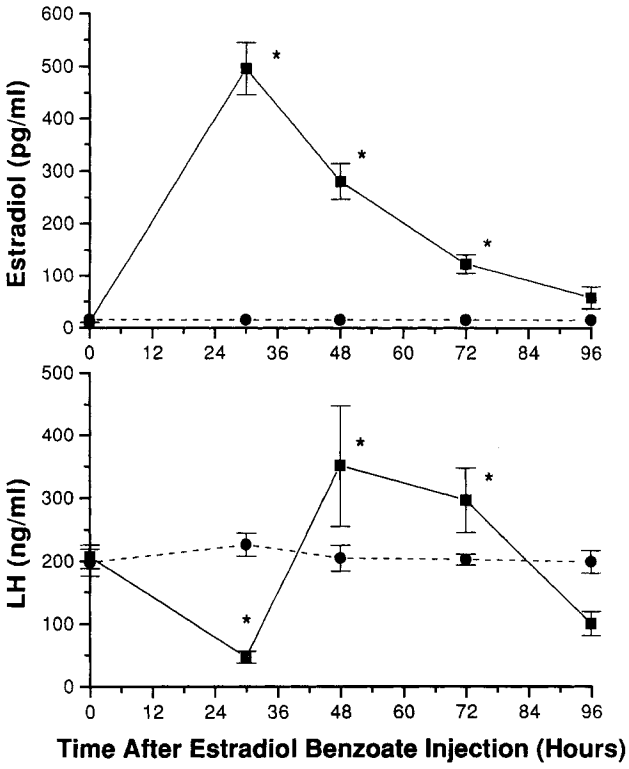

Fig. 3. Pattern of mean (± SEM) serum concentrations of E_2 and LH in blood samples taken on times 0 hr, 48 hr, 72 hr, and 96 hr in ovariectomized cynomolgus monkeys. The solid line represents data when the monkeys were treated with one subcutaneous injection of estradiol benzoate (E_2B) (50 μg/kg) at time 0 hr. The dotted line represents control data without E_2B treatment. (*) denotes a significant difference ($P < 0.05$) from the values observed on time 0 hr.

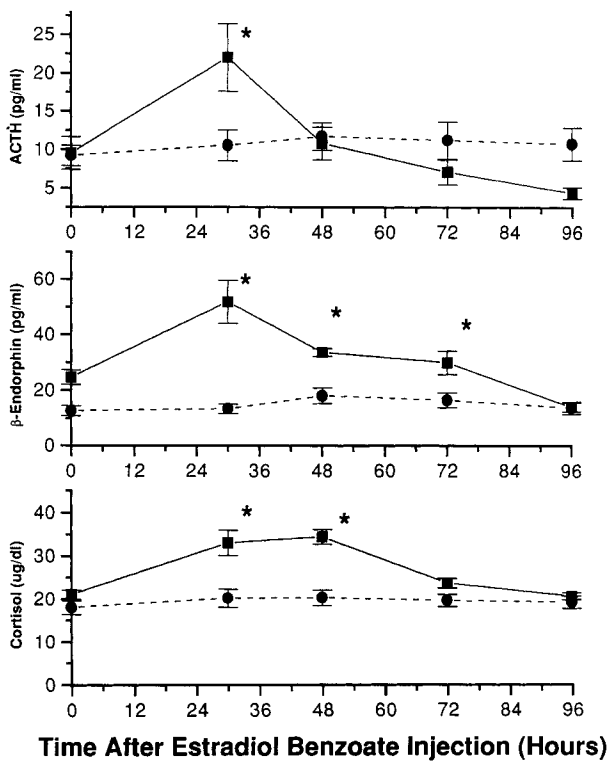

Fig. 4. Pattern of mean (± SEM) serum concentrations of ACTH, β-endorphin, and cortisol in blood samples taken on times 0 hr, 48 hr, 72 hr, and 96 hr in ovariectomized cynomolgus monkeys. The solid line represents data when the monkeys were treated with one subcutaneous injection of estradiol benzoate (E_2B) (50 μg/kg) at time 0 hr. The dotted line represents control data without E_2B treatment. (*) denotes a significant difference ($P < 0.05$) from the values observed on time 0 hr.

(ventricular) administration of opiate peptides leads to decreased release of LH and FSH (Ferin et al., 1984). It is generally agreed that GnRH neurons lack receptors for E_2. However, β-EP neurons do have E_2 receptors (Jirikowski et al., 1986) and β-EP terminals are present on GnRH neurons (Bicknell, 1985). It is also generally agreed that β-EP has no direct pituitary effect on the secretion of gonadotropins. Thus, β-EP may inhibit gonadotropin secretion either directly by preventing the release or synthesis of GnRH or indirectly by affecting the presynaptic release of some unknown stimulator or inhibitor of GnRH release. On the other hand, it has long been known that activation of the CRH-ACTH-cortisol axis by a variety of stimuli such as stress (Rivier et al., 1986) or interleukin-1 (Feng et al., 1991) inhibits LH release.

In the AP, CRH content was highest at 72 hr post-E_2B. We speculate that this increased AP content of CRH at 72 hr probably results from the release of CRH from the HT as reflected in low levels seen there at 48 hr. The significant decrease in HT CRF content seen 48 hr post E_2-B was not accompanied by the expected changes in AP ACTH content. The reason for this remains unclear. However, it is possible that the sampling frequency was insufficient to detect a short-term effect. It has been clearly demonstrated that the rapid inhibitory effect on LH is an action of CRH (Rivier et al., 1986; Olster and Ferin, 1987; Petraglia et al., 1987; Williams et al., 1990; Feng et al., 1991) and not of ACTH or cortisol since intravenous infusion of ACTH to ovariectomized monkeys does not lead to the same decrease in LH secretion as CRH does (Xiao and Ferin, 1988). Furthermore, CRH decreases plasma LH levels by inhibiting GnRH release into the hypophyseal-portal blood in female rats (Petraglia et al., 1987) and CRH injected bilaterally into the medial preoptic area (MPOA) suppressed hypothalamic GnRH release into the median eminence, an effect which does not occur when CRH was injected into the paraventricular nucleus of the hy-

pothalamus or into the median eminance in proestrus rats (Rivier et al., 1986). Interestingly, the suppressive effect of CRH can be partially reversed by a previous administration of opioid mu or mu1-receptor antagonists (Rivest et al., 1993), suggesting that the inhibitory action of CRH can be a direct one, or mediated through the release of β-EP. Additionally, direct connections exist between CRH axons and GnRH neurons in the MPOA (MacLusky et al., 1988) and interactions between vasopressin and GnRH neurons have been documented in the monkey supraoptic nucleus (Thind et al., 1991). Furthermore, it remains to be determined whether the CRH, ACTH and β-EP system operating at the AP level is of any physiological importance, in the control of the E_2B-induced LH surge.

We acknowledge that 1) the methodology used in this experiment (only one structure content evaluated everyday) is certainly not sufficient for a precise determination of time-dependent causal correlations between structure contents and plasma hormonal levels, and 2) the structure contents (a complex equilibrium between synthesis and release) do not always accurately reflect variations in hormone release (which vary in a circadian and/or pulsatile fashion for the hormones measured herein). However, the results presented here show that there may be activation of the hypothalamo-anterior pituitary-adrenal axis during the negative and the positive feedback phases of the E_2B-induced LH surge in these OVX primates. To date, we know of no studies demonstrating an activation of the hypothalamus-pituitary-adrenal axis in intact cynomolgus monkeys at mid-cycle. However, the human data (Genazzani et al., 1975) suggests that this may occur. Whether this is important for the timely initiation and/or full development of the LH surge in the primate ovulatory menstrual cycle remains to be seen.

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