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Physiology

Estradiol and the inhibition of hypothalamic gonadotropin-releasing hormone pulse generator activity in the rhesus monkey

(single-unit activity/desynchronization/luteinizing hormone surge)

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ABSTRACT **In mammals, gonadal function is controlled by a hypothalamic signal generator that directs the pulsatile release of gonadotropin-releasing hormone (GnRH) and the consequent pulsatile secretion of luteinizing hormone. In female rhesus monkeys, the electrophysiological correlates of GnRH pulse generator activity are abrupt, rhythmic increases in hypothalamic multiunit activity (MUA volleys), which represent the simultaneous increase in firing rate of individual neurons. MUA volleys are arrested by estradiol, either spontaneously at midcycle or after the administration of the steroid. Multiunit recordings, however, provide only a measure of total neuronal activity, leaving the behavior of the individual cells obscure. This study was conducted to determine the mode of action of estradiol at the level of single neurons associated with the GnRH pulse generator. Twenty-three such single units were identified by cluster analysis of multiunit recordings obtained from a total of six electrodes implanted in the mediobasal hypothalamus of three ovariectomized rhesus monkeys, and their activity was monitored before and after estradiol administration. The *bursting* of all 23 units was arrested within 4 h of estradiol administration although their baseline activity was maintained. The bursts of most units reappeared at the same time as the MUA volleys, the recovery of some was delayed, and one remained inhibited for the duration of the study (43 days). The results indicate that estradiol does not desynchronize the bursting of single units associated with the GnRH pulse generator but that it inhibits this phenomenon. The site and mechanism of action of estradiol in this regard remain to be determined.**

In all vertebrates studied in this regard, reproduction is totally dependent on the normal functioning of a hypothalamic signal generator that governs the pulsatile release of gonadotropin hormone-releasing hormone (GnRH), a neuropeptide that stimulates the secretion of the pituitary gonadotropic hormones (see ref. 1 for review). In the rhesus monkey, this central oscillator, generally referred to as the GnRH pulse generator, has been localized to the area of the arcuate nucleus (2), but its neuronal composition and supracellular organization remain in doubt (3).

The activity of the GnRH pulse generator can be assessed by detecting pulses of luteinizing hormone (LH) in peripheral blood, by measuring pulsatile release of GnRH into the extracellular fluid of the hypothalamus and the pituitary gland, or by monitoring the electrical changes that accompany these neurosecretory processes (see ref. 4 for review). These consist of abrupt increases in multiunit activity (MUA volleys) recorded from the mediobasal hypothalamus. The invariable association between these MUA volleys and the initiation of LH pulses (Fig. 1) observed under a variety of experimental circumstances in the rhesus monkey (5–12), rat (13–18), and goat (19–21) has led to the conclusion that these electrical signals indeed represent the electrophysiological manifestations of GnRH pulse generator activity.

Multiunit recordings, however, can provide only a measure of total neuronal activity and provide no insight into the behavior of individual neurons. Recently we have described the activity of single units associated with the GnRH pulse generator in female rhesus monkeys by using a spike analysis program that has revealed that the MUA volleys represent the simultaneous increase in firing of a subset of individual hypothalamic units and a decrease in frequency of a smaller population of others (22).

Radiotelemetric monitoring of MUA associated with GnRH pulse generator activity in freely behaving normal rhesus monkeys revealed a dramatic inhibition of this activity at midcycle that appears to be occasioned by the rise in serum estradiol that also initiates the preovulatory LH surge (11). To examine whether this arrest of the MUA volleys by estradiol reflects a similar inhibition of the bursts of the underlying single units or some other phenomenon such as their desynchronization, we have utilized the same spike analysis method (22) to identify and monitor the activity of hypothalamic single units associated with GnRH pulse generator activity before and after the administration of estradiol and throughout the resulting surge of LH. A preliminary report of this work has been presented.‡

MATERIALS AND METHODS

Three adult, long-term ovariectomized (9–70 months) rhesus monkeys (*Macaca mulatta*, 7.1–8.8 kg) were studied. They were housed singly in temperature- and light-controlled rooms (0700–1900 h light phase) and fed once daily with Purina Monkey Chow (Ralston Purina) or High Protein Monkey Diet (PMI Feeds, St. Louis) supplemented with fresh fruit thrice weekly. Water was available ad libitum. The animals were fitted with bilateral chronic recording electrode arrays, each consisting of nine 50-μm insulated Nichrome wires (California Fine Wire, Grover City, CA) implanted stereotaxically in the mediobasal hypothalamus 5–70 months before the experiments (23). The electrodes were screened for the characteristic increases in MUA associated with the initiation of each pulse of LH as measured in peripheral blood.

During recording sessions, the animals were restrained in primate chairs to which they had been extensively habituated.

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Abbreviations: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; MUA, multiunit activity; EB, estradiol benzoate.

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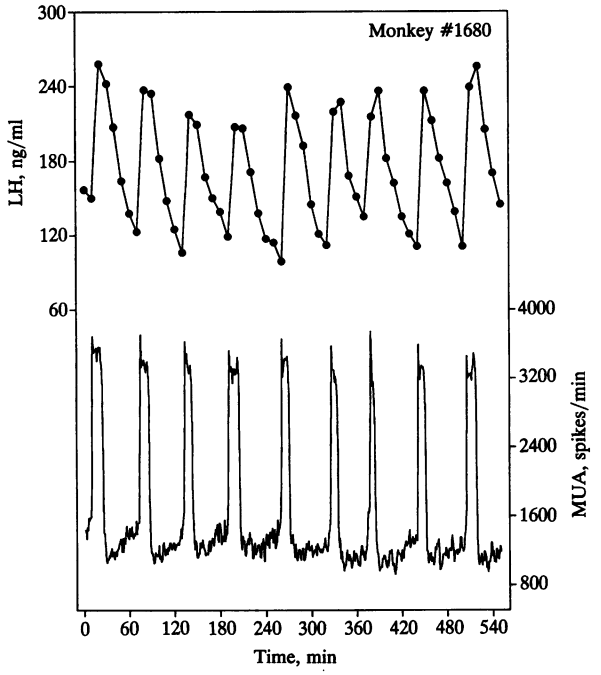

FIG. 1. MUA volleys recorded from the mediobasal hypothalamus and LH pulses measured in the peripheral circulation of an ovariectomized rhesus monkey (reproduced from ref. 22).

In each animal, recordings were performed from two electrodes against a common reference electrode that had been previously identified as "inactive" in terms of MUA associated with pulse generator activity. The electrodes were connected through high-impedance probes (model CFP-1020; BAK Electronics, Clarksburg, MD) and high-input-impedance modules (model H1P511E; Grass Instruments, Quincy, MA) to P511 preamplifiers (Grass Instruments) to provide signal amplification (×36,000 to ×120,000) and filtering (300- to 1000-Hz bandpass) (23). The analog output was digitized by the DISCOVERY Version 3.2 software (DataWave Technologies, Longmont, CO) at a sampling rate of 32 or 18 kHz, and the spikes were extracted when their positive phase exceeded an arbitrary threshold (22). In addition, the analog signal was analyzed with the DATAQUEST III software (Data Sciences, St. Paul) after amplitude discrimination (WPI model 121; W-P Instruments, New Haven, CT) to obtain on-line MUA histograms as described (23). The extracted single-unit discharges were separated off-line by the COMMON PROCESSING PACKAGE Version 3.2 software (DataWave Technologies) using cluster analysis as described (22). This analysis was performed on a portion of the signal that had been obtained during an MUA volley before estradiol benzoate (EB) administration (see below). The cluster boundaries specified for any one electrode were saved on computer files and used for the entire study.

The waveforms attributable to the firing of single units were easily distinguished from others by their shapes (22). Only animals that had at least one electrode with at least two unambiguously separable units associated with the GnRH pulse generator as indicated by the presence of bursts with onsets and terminations invariably synchronous with those of the MUA volleys (22) and with at least one unit unrelated to the activity of the pulse generator were included. Of the 21 ovariectomized monkeys screened, 3 met the above criteria and were selected for the study. In most of the remaining animals (16 of 18), only one waveform per electrode was found or no reliable separation of the units could be achieved, probably because of the distances between the active units and the recording electrodes (22). In one animal, no single unit unrelated to the activity of the GnRH pulse generator was found, whereas in the remaining monkey the extracted units were lost before the start of the experiment.

The experimental design employed is illustrated in Fig. 2. In all three monkeys, EB was given subcutaneously (84 μg/kg dissolved in peanut oil) between 1400 and 1415 h on the first day of the experiment. On this day, MUA was recorded for 4–5.5 h before (control) and 5–6.5 h after the injection. On days 2, 3, and 4, recordings were performed at 0800–2000 h (ascending limb of the LH surge), 0800–1730 h (peak of the LH surge), and 0930–1530 h (descending limb), respectively (8, 24) (Fig. 2). On days 1–3, recording was interrupted for 5–15 min between 1300–1415 h to administer the injection, to take blood, and to change computer files. After the LH surge, the recovery of MUA volleys and single-unit bursts was monitored once a week up to day 43.

Daily blood samples were taken by femoral puncture for 5 days before the experiment, three times daily on days 1 and 2, twice daily on days 3 and 4, and once a week between days 7 and 43. Serum concentrations of LH were measured by bioassay (11), and concentrations of estradiol were measured by a commercially available double-antibody RIA kit (Diagnostic Products, Los Angeles).

RESULTS

Thirty-five single units (3–10 per electrode) were identified in multiunit signals recorded from a total of six electrodes implanted in the mediobasal hypothalamus of the three ovariectomized rhesus monkeys. Their activity could be monitored throughout the entire study (36–43 days). Twenty-three of the 35 units displayed increases in activity with onsets and terminations invariably synchronous with those of the MUA volleys, indicating that they were associated with the GnRH pulse generator (22). The activity of the remaining 12 neurons did not change in relation to the MUA volleys. In the present study, no units with decreased activity during the MUA volleys (22) were found.

Peak serum concentrations of estradiol (1821–2275 pg/ml) occurred at 5.5–23.5 h after EB administration. EB simultaneously arrested the bursting of all 23 units associated with

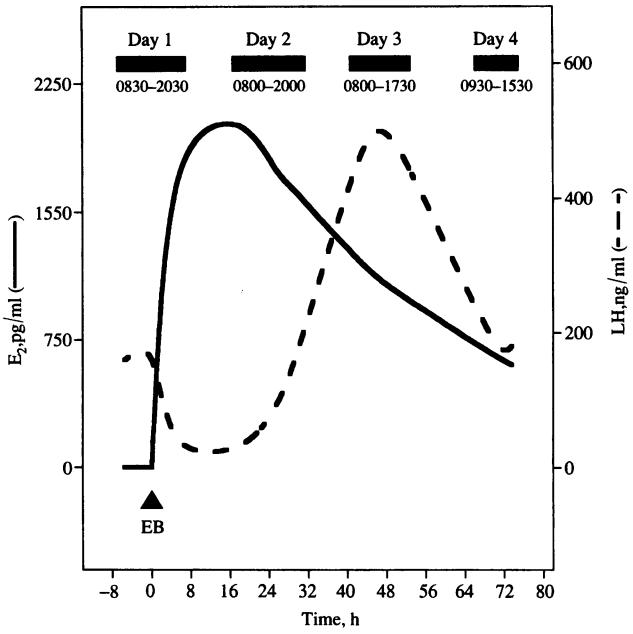

FIG. 2. Diagrammatic representation of the periods of electrical recordings (horizontal bars) performed on the first 4 days of the experiments in relation to the changes in serum estradiol (E_2) and LH concentrations after EB administration on day 1 between 1400 and 1415 h (time 0).

pulse generator activity within 2–4 h without inhibiting their basal firing rate. The increase in serum estradiol concentrations also evoked an LH surge in each animal with peak serum levels of 225–630 ng/ml occurring at 42–52 h after the injection.

The recovery of GnRH pulse generator activity was first detected 7–22 days after EB administration, as indicated by the recurrence of MUA volleys and the underlying bursts of most (15 of 23) single units, albeit at reduced frequencies, amplitudes (firing rate), and durations when compared to the preinjection period. Although serum estradiol levels returned to preinjection levels 15–22 days after the injection, the recovery of these parameters continued beyond this point of time. The bursting of the remaining (8 of 23) units associated with the pulse generator was still inhibited when the MUA volleys recurred, although their baseline activity was maintained. Subsequent recordings revealed that the recovery of the bursting of 7 of these units was delayed by 1–3 weeks, and one remained inhibited for the duration of the study (43 days). The results of a representative experiment are shown in Fig. 3.

DISCUSSION

Our findings indicate that in the ovariectomized rhesus monkey, the dramatic inhibition of the hypothalamic MUA volleys, which takes place within 2–4 h after EB administration (8), is due to the simultaneous inhibition of the bursting activity of individual units (e.g., Fig. 3, units 1–3, days 1–4) and cannot be accounted for by the desynchronization of the single-unit bursts as suggested by some (25, 26). The synchrony between the onsets and terminations of these bursts was also maintained when the MUA volleys reappeared 7–22 days after EB administration. Consequently, the gradual increases in the frequency and duration of the MUA volleys observed during the recovery period were faithfully reflected by similar changes in these parameters of the bursts of the single units (e.g., Fig. 3, MUA and units 1 and 2, days 7 and 22). These results not only reinforce our earlier conclusion that the MUA volleys represent the sum of sharp, synchronous changes in activity of individual units (22) but also indicate that their disappearance reflects the uniform inhibition of the underlying single-unit bursts, at least under circumstances characterized by high ambient levels of estradiol.

The single units associated with the GnRH pulse generator, however, do not always display the same pattern of activity. The gradual increase in the amplitude (i.e., firing rate within the bursts) of the MUA volleys during the period of recovery from estradiol-induced inhibition was due only in part to the increase in the amplitude of the single-unit bursts. In addition to this mechanism, the *number* of neurons displaying bursting activity also increased with time (e.g., Fig. 3, unit 3, days 7 and 22), indicating that these cells might not be equally sensitive to the effects of estradiol.

The mechanisms by which estradiol inhibits the bursting activity of the single units associated with the GnRH pulse generator remain unknown. A compelling body of evidence suggests that in higher primates and rodents the inhibition of the GnRH pulse generator by estradiol is mediated, at least in

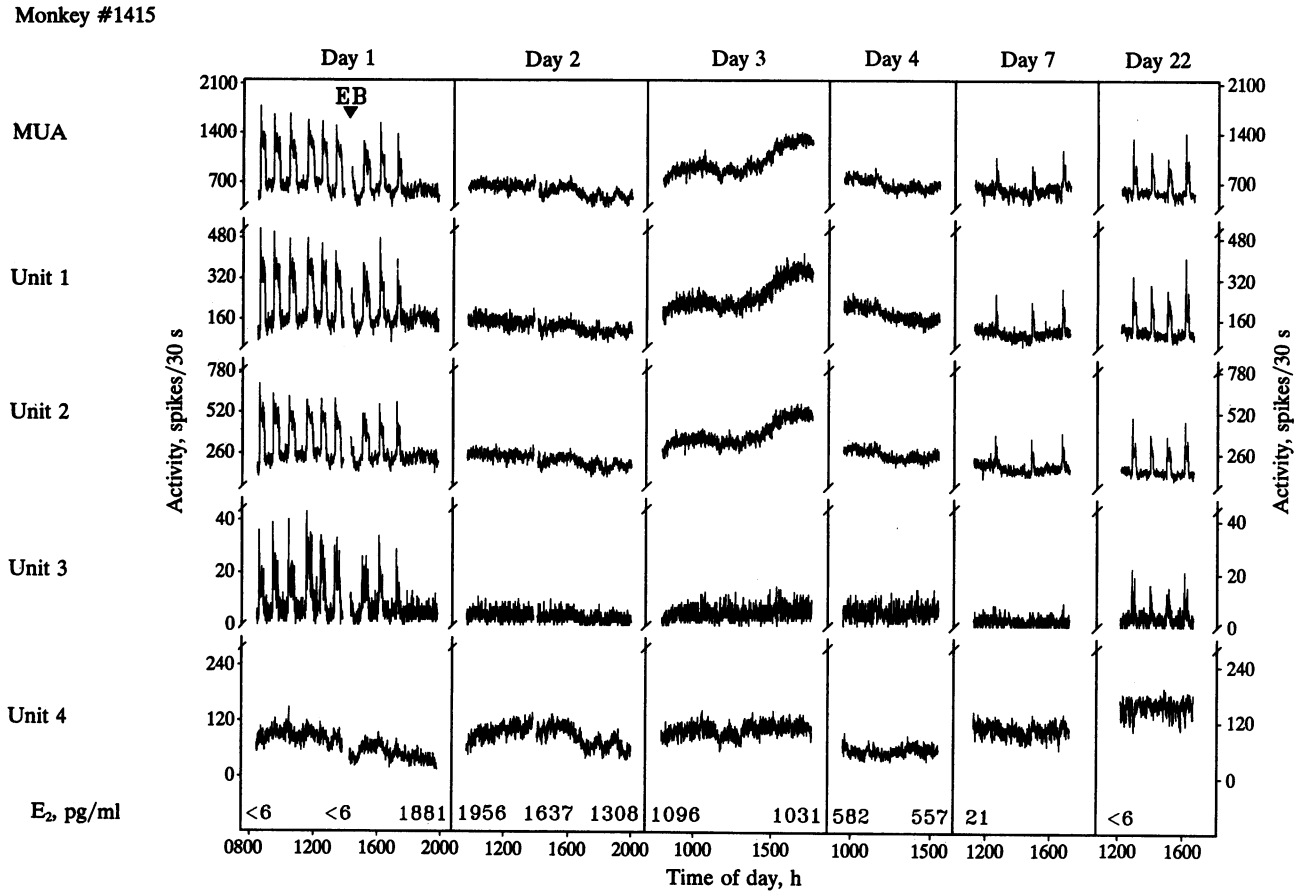

FIG. 3. Time-based histograms of MUA (top trace) and of 4 units extracted from this multiunit signal recorded from the mediobasal hypothalamus of a representative ovariectomized rhesus monkey. Serum estradiol (E_2) concentrations are shown at the bottom of the figure. Before EB administration (at 1400 h on day 1), units 1–3 displayed increases in activity that were invariably synchronous with the MUA volleys (top line), whereas the activity of unit 4 (bottom line) was unrelated to them. EB injection resulted in a synchronous inhibition of bursts of single units coincident with the cessation of MUA volleys at 1800 h on day 1, which was maintained for at least 4 days. Note that on day 7, when the MUA volleys and the bursts of units 1 and 2 recurred, unit 3 had not recovered its control activity.

part, by endogenous opioid peptides (16, 27–29). Although it has been suggested that opioids may exert their inhibitory effects by hyperpolarizing the membranes of their target neurons in the arcuate nucleus (30), the undiminished baseline firing of the units whose bursting activity has been inhibited (e.g., Fig. 3, units 1–3, day 1) does not support such an explanation. Moreover, the gradual recovery of the amplitude and duration of the single-unit bursts and of the resultant MUA volleys continued and was still not complete 7–20 days after estradiol had returned to preinjection levels. This finding, which is very similar to that observed after ovariectomy (31), is inconsistent with an opioid mediation of the steroid-induced inhibition and suggests that the protracted effects of exogenous or endogenous estrogen withdrawal may reflect a structural remodeling in the hypothalamus (32–34) as discussed in detail elsewhere (31).

Although the peak serum concentrations of estradiol were much greater in the present study than those measured in gonadally intact rhesus monkeys in the late follicular phase of the menstrual cycle [≈2000 pg/ml vs. ≈300 pg/ml respectively (11, 35)], there is no reason to believe that the underlying mechanisms are not the same. Clearly, however, the higher estradiol levels completely inhibited GnRH pulse generator activity for a much longer time, 7–22 days in this study, whereas MUA volleys reappear within 24–48 h during the normal menstrual cycle (11).

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