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Duration of phasic electrical activity of the hypothalamic gonadotropin-releasing hormone pulse generator and dynamics of luteinizing hormone pulses in the rhesus monkey

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ABSTRACT **The secretion of luteinizing hormone (LH) by the pituitary gland is a pulsatile phenomenon. In the rhesus monkey, each pulse of LH in the peripheral circulation is associated with a characteristic increase in multiunit electrical activity (MUA) recorded from the medial basal hypothalamus. These "volleys" of electrical activity initiate the release of gonadotropin-releasing hormone (GnRH) into the pituitary portal circulation from the terminals of neurosecretory cells. Their duration varies from 1–3 min in normal, adult intact females to 10–25 min in long-term ovariectomized monkeys. A variety of pharmacological interventions also modify volley duration. The purpose of this investigation was to determine the physiological significance of alterations in volley duration. The dynamics of LH pulses in ovariectomized animals were observed in a number of experimental circumstances in which MUA volley duration was reduced from a maximum of 23 min to a minimum of 4 min without significantly altering their frequency. The magnitude of each LH pulse was assessed by calculating the area under the curve delineated by the time course of LH above baseline. In eight experiments, a linear regression of these values on volley duration failed to reveal a significant correlation between MUA volley duration and the magnitude of LH pulses. These results suggest that all of the GnRH secreted per pulse is released at the onset of each MUA volley, the remainder of the increase in electrical activity having no further action on GnRH secretion, although effects on other systems cannot be excluded.**

The secretion of hypophysial gonadotropic hormones in all vertebrates studied to date is a pulsatile phenomenon controlled by a central pattern generator that triggers the release of the hypothalamic peptide gonadotropin-releasing hormone (GnRH) into the pituitary portal circulation (for brief reviews, see ref. 1). The electrophysiologic correlates of GnRH pulse generator activity in the rhesus monkey have been described in extenso (2–4). In the ovariectomized animal, they are characterized by abrupt increases in multiunit electrical activity (MUA) that consist of a brief "overshoot" followed by a plateau phase that ends in a rapid decline to baseline activity. In such monkeys, MUA volleys occur every 50–60 min and persist for 10–25 min. The onset of each MUA volley is associated with the initiation of a pulse of luteinizing hormone (LH) in the peripheral circulation. In intact animals, the MUA volley is essentially limited to the overshoot and persists for 1–3 min (5). Estradiol given to ovariectomized monkeys reduces the duration of MUA volleys to that characteristic of intact animals (4), as does morphine (3, 6) and corticotropin-releasing factor (CRF) (7), while pentobarbital anethesia results in increased MUA volley duration (2).

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The aim of this investigation was to determine the relationship between the duration of increased electrical activity and GnRH release as estimated by the dynamics of LH pulses. Such an analysis is potentially complicated by the inverse relationship between frequency and LH pulse amplitude (8) and by the fact that most experimental manipulations that modify MUA volley duration also modify their frequency (2–4) and must, therefore, be limited to experimental circumstances in which MUA volley duration is varied without altering MUA volley frequency.

METHODS

Four long-term ovariectomized rhesus monkeys fitted with bilateral arrays of recording electrodes in the medial basal hypothalamus and with chronic cardiac catheters were used in this study. On the day of the experiment, they were placed in primate chairs and hypothalamic MUA was recorded for 6–10 hr while blood samples were taken at 10-min intervals for the measurement of serum LH concentration by radioimmunoassay. The details of the foregoing techniques and procedures have been described (2).

In eight experiments, MUA volley duration was reduced without significant change in MUA volley frequency by the administration of CRF and morphine and by other interventions (6). MUA volley duration was measured from the time of the onset of the volley to the time when MUA had returned to baseline. The interval between volleys (1/frequency) was taken as the time from the initiation of one volley to that of the next.

The area under each LH pulse (ng·min·ml^{-1}), defined from nadir to nadir, was calculated by the trapezoidal rule. A multiple regression analysis of LH area on MUA volley duration, interval, and differences between monkeys was performed, and the partial correlation between MUA volley duration and LH pulse area was evaluated by adjusting the area to factor out the influences of interval and monkey differences (9).

RESULTS

Fig. 1 is an example of an experiment in which MUA volley duration was significantly reduced from 13.5 ± 3.7 min (n =

Abbreviations: GnRH, gonadotropin-releasing hormone; MUA, multiunit electrical activity; LH, luteinizing hormone; CRF, corticotropin-releasing factor.

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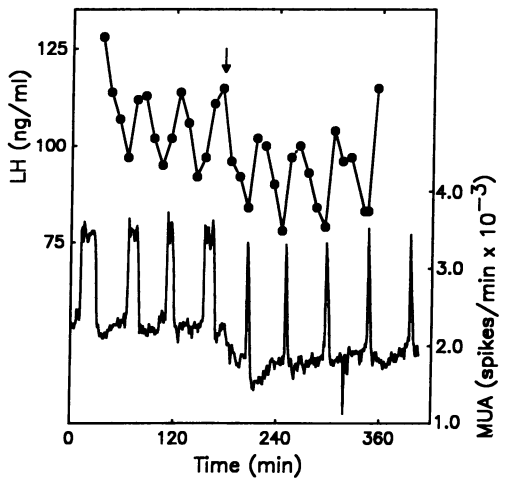

FIG. 1. A representative experiment illustrating the relation between hypothalamic MUA volleys and LH pulses in the peripheral circulation of an ovariectomized monkey. CRF (100 μg) was administered i.v. at the arrow. There was a dramatic decrease in the duration of the subsequent MUA volleys (lower trace) without a change in the magnitude and dynamics of the LH pulses (upper trace). The decline in baseline electrical activity following CRF injection does not bear on the analysis.

4) before CRF administration to 4.6 ± 0.5 min (n = 5; mean ± SD; $P < 0.01$) after treatment without a change in MUA volley frequency (interval between volleys, 47.7 ± 6.4 vs. 48.0 ± 2.0 min).

The data from eight such experiments were pooled, and the adjusted area under each pulse of LH was plotted against the duration of its corresponding MUA volley. This partial correlation (see *Methods*) is shown in Fig. 2. There was no relation between MUA volley duration and the area under the corresponding LH pulse (n = 56 pulses; r = 0.123; P = 0.89 by analysis of variance). Similarly, there was no apparent relation between MUA volley duration and the dynamics of the LH pulses.

DISCUSSION

The results of this study lead to the conclusion that major changes in the duration of the volleys in electrical activity associated with the operation of the hypothalamic GnRH pulse generator do not result in significant alterations in the magnitude or dynamics of the resultant LH pulses. A correlative conclusion is that the entire effective bolus of GnRH that initiates each pulse of LH is discharged at the onset of increased neuronal activity, the remainder of the period of accelerated firing having no additional effect on the secretion of the neuropeptide. This conclusion is based on the following considerations:

(*i*) There is a linear relation between the quantity of LH released by gonadotropes, either *in vitro* (10, 11) or *in vivo* (12, 13), and the quantity of GnRH administered acutely. The concentrations of GnRH achieved in these studies fell within the range measured in pituitary portal blood of ovariectomized monkeys (14–16), humans (16), ovariectomized ewes (17), and rats (18) or in the extracellular fluid of the medial basal hypothalamus of rats (19) and sheep (20) sampled by "push–pull" perfusion.

It follows that if, in our study, MUA volleys of longer duration had resulted in increased secretion of GnRH than the shorter volleys, LH pulses of higher amplitude would have been observed. Such was not the case.

(*ii*) Increasing the duration or "width" of the GnRH stimulus to pituitary cells *in vitro*, its magnitude and frequency remaining constant (10), results in LH pulses of longer duration. Similarly, in ovariectomized ewes, constant amounts of GnRH given over increasing periods of time (from a 15-sec i.v. bolus to an 18-min infusion) produced progressive reductions in amplitude of the resulting LH pulses and delays in the peak LH concentration achieved (21). It would seem, therefore, that if GnRH secretion had continued at a constant rate throughout the duration of the MUA volleys, the longer volleys would have resulted in LH pulses with dynamics quite different from those observed in LH pulses associated with the shorter MUA volleys. Again, such differences were not observed.

The conclusion that the effective bolus of GnRH is released at the very onset of each MUA volley, the remainder of increased electrical activity having no further action in hormone release, is consonant with the findings of Handelsman *et al.* (21) that a single bolus of GnRH, but a few seconds in duration, is the most effective stimulus pattern for eliciting maximal LH responses in ovariectomized ewes.

Whereas volley duration does not appear to be involved in the regulation of LH secretion, it may be of consequence in the control of other systems. It is tempting to speculate, for example, that because ovariectomy in the monkey can prolong MUA volley duration 10-fold (5), an effect that is reversed by estradiol (4), the "hot flushes" of ovarioprivic women, profound vasomotor disturbances synchronous with LH pulses (22), may be occasioned by a prolongation of the enhanced electrical activity of the GnRH pulse generator.

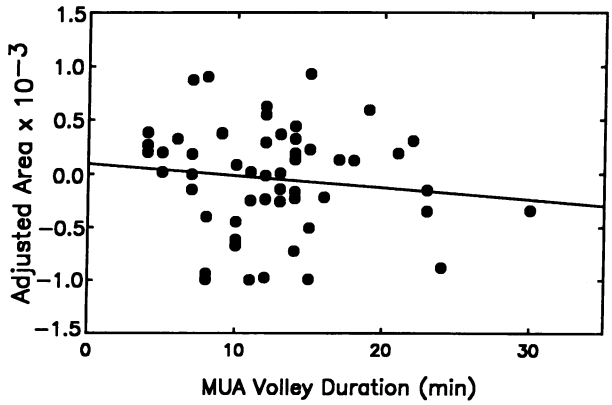

FIG. 2. A partial correlation of hypothalamic MUA volley duration on adjusted area under each LH pulse from eight experiments such as the one shown in Fig. 1. The adjusted area units were derived from the original data for each animal. There was no relation between the MUA volley duration and the magnitude of the corresponding LH pulse (n = 56 pulses; r = 0.123; P = 0.89).

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