PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 64–68 (1975)

Rhythmic Patterns of Endogenous LH Release in Castrate Sheep Receiving Exogenous LH[1] (38477)

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Motta *et al.* (1) have summarized much of the evidence indicating that regulation of anterior pituitary (AP) hormone release by the hypothalamo–hypophysial unit involves the "short-loop" feedback of AP hormones to influence their own secretion. However, most of this evidence involved prolonged administration of AP hormones. Improved assay procedures for plasma hormones, combined with short-interval blood sampling, have revealed episodic fluctuations in many AP hormones. There are only limited data on whether the temporary elevation of circulating AP hormone levels, as occurs normally, can inhibit subsequent pituitary release by a short-loop feedback mechanism. Sakuma and Knobil (2) reported that elevation of plasma growth hormone (GH) by exogenous administration inhibited both insulin-induced and vasopressin-induced release of endogenous GH in monkeys. On the other hand, Tucker, *et al.* (3) reported that elevated levels of plasma prolactin (PRL) in cows did not reduce the milking-induced release of pituitary PRL. Toft *et al.* (4) also failed to observe any effect of exogenous thyroid-stimulating hormone (TSH) on the release of endogenous TSH in man.

When studied by sequential short-interval sampling, fluctuating patterns of plasma luteinizing hormone (LH) in castrate animals exhibit a regular period and can be classified as rhythmic (5–8). The mechanisms regulating these rhythmic discharges of pituitary LH are incompletely understood. Administration of exogenous estrogen abolished the rhythmic LH pattern in castrate monkeys (9) and sheep (10). The temporal pattern of plasma LH in untreated castrates was suggestive of a regulatory mechanism involving short-loop feedback of circulating LH to govern the timing of subsequent LH discharges from the pituitary. The present research investigated the possible existence of a mechanism by which elevated plasma LH resulting from each pulsatile discharge inhibited the next discharge until plasma LH had declined to low levels.

Materials and Methods. Eight crossbred sheep (40–50 kg body wt) were ovariectomized at 6–9 mo of age and plasma LH studies were begun 1–4 mo later. Each ovariectomized ewe required two indwelling vascular cannulae, one in each jugular vein, so that blood sampling and hormone infusions could be accomplished independently. Each of the 14 experimental replicates consisted of one control (4 hr) and one experimental (6 hr) sampling sequence on consecutive days during which heparinized blood samples were collected at 10-min intervals. Intravenous infusion of ovine LH (NIH-S16 or S18) via one jugular vein cannula was begun at the start of each experimental sequence and continued throughout. Ovine LH was infused at a rate of 0.3 mg/hr (2 trials) or 0.5 mg/hr (12 trials) and was dissolved in a saline solution containing 1 % egg white. At the start of one preliminary experimental sequence, a priming dose of 0.5 mg LH was administered.

LH was quantified in all plasma samples by radioimmunoassay (RIA) procedures using the No. 573 antiserum as reported previously (10). This RIA accurately quantified LH only in the range of 0.2–8.0 ng of standard LH (NIH-S16) per assay tube. Plasma samples collected during control sequences were assayed in duplicate with either 100 or 200 μl plasma per assay tube. Plasma obtained during LH infusion contained very high levels of immunoreactive LH and each assay tube could only contain a small volume of this plasma without exceeding the upper limit of the LH measurement range. The procedure of pipetting these

[1] Journal paper No. 5528, Purdue University Agricultural Experiment Station. Research supported in part by PHS Grant No. HD 5114.

small volumes directly was considered inappropriate because of the errors involved. Therefore, these plasma samples were diluted 1:5 with assay diluent and duplicate aliquots of 50, 100 and 200 μl of diluted sample were assayed. The six estimates of unknown LH concentration for each sample were averaged prior to statistical analysis.

Data evaluation. Statistical analysis of fluctuating patterns of plasma LH as determined by sequential short-interval sampling, must include recognition of nonrhythmic, and rhythmic fluctuations. The following two questions needed to be answered about each sequence (time series) of plasma LH measurements. (A) Did the fluctuations in mean plasma LH for sequential 10-min samples exceed the RIA variability as estimated from the within-sample variance? (B) If plasma LH did in fact fluctuate, were the fluctuations rhythmic (i.e., possessing a regular period)?

The method of answering question A for each control sequence was a straightforward analysis of variance (ANOV) in which the within-sample variance (i.e., RIA variability) was compared with the variance among samples (independent of their sequential nature). When considering the experimental sequences, plasma LH increased dramatically during the first hour of LH infusion and the normal application of the ANOV procedure would invariably show that the among-sample variance was much greater than the within-sample variance. It was therefore necessary to remove from the normal estimate of the among-sample variance that variance which resulted from the infusion of LH. A curvilinear regression line was fitted by least squares techniques to the time series of plasma LH from each experimental sequence. It was assumed that this fitted line reflected the exogenous LH infused plus an integrated average of endogenous LH. Deviations of actual plasma LH at each 10-min interval from the fitted line were used as the best estimate of acute changes in endogenous LH release. After converting all the data for experimental sequences to such deviations, ANOV comparisons of within-sample and among-sample variances were performed.

The determination of rhythmicity (question B above) was accomplished by power spectral analysis as previously described (10, 11). In addition to analyzing all control and experimental time series of plasma LH for rhythmicity, the deviation data for experimental sequences derived as described in the previous paragraph were subjected to the spectral analysis. When comparing by power spectrum the various frequency components contained within an individual sampling sequence, the frequency with the largest power value is considered as the major component. Because the determinations of rhythmicity and of major frequency components were so critical to interpretation of the present results, an objective system of classifying power spectra was adopted (12). The system compared the largest power value against the mean and standard deviation (SD) of all lower power values for that particular time series. The difference between the largest power value and the mean of all lower values was divided by the SD calculated from the lower values. When this ratio exceeded three, the time series was classified as extremely rhythmic. Time series with ratios between two and three were classified as moderately rhythmic, and those with ratios less than two were considered nonrhythmic.

Results and Discussion. The infusion of ovine LH into ovariectomized sheep during the experimental sampling sequences increased the mean plasma concentration of LH from 6.6 to 123 ng/ml (Table I). The RIA variability, as estimated from the within-sample variance, increased proportionately so that the within-sample coefficients of variation were 18% and 14%, respectively, for the control and experimental sequences. LH infusion did not abolish the plasma LH fluctuations. In fact, the results of the ANOV procedure, applied to the deviation data as described previously, indicated that significant LH fluctuations were present in 100% of the experimental sequences (Table I).

Figure 1 presents one of the experimental sequences best illustrating the continued fluctuation of plasma LH during LH infusion. In the lower graph, the actual plasma LH concentrations for the 10-min samples were plotted together with the fitted curvilinear regression line. In the upper graph (Fig.

TABLE I. COMPARISON OF PLASMA LH PATTERNS IN CONTROL AND EXPERIMENTAL SEQUENCES.

Comparison	Control	Experimental	
	Actual data	Actual data	Deviation data
Number of trials	14	14	14
Plasma LH parameters:			
Mean concentration (ng/ml)	6.6	123	—
Within-sample coefficient of variation	18%	14%	—
Percent of trials in which among-sample variance exceeded within-sample variance ($P < 0.01$)	86	—	100
Spectral analysis:			
Percent of trials classified:			
Extremely rhythmic	43	79	72
Moderately rhythmic	29	7	7
Rhythmic	71	86	79
Mean period (min) for those extremely rhythmic frequencies[a]	60	59	50

[a] In this calculation those periods ≥106 min in the experimental sequences were excluded because they could not have been detected in the shorter control sequences.

1), the deviations from the regression line were plotted in order to illustrate this procedure and the rhythmic nature of the deviations. It was assumed that these deviations resulted from variable release of endogenous LH although abrupt changes in the rate of removal of exogenous LH from plasma cannot be excluded.

The determinations of rhythmicity for time series of plasma LH concentrations used the spectral analysis procedures described previously. Among control trials 71 % of the plasma LH patterns were classified as either moderately or extremely rhythmic (Table I). Application of the same classification system to the actual and deviation data from experimental sequences indicated that 86 % and 79 %, respectively, were rhythmic. It was noteworthy that among the experimental sequences there was a larger percentage of extremely rhythmic patterns. This difference cannot presently be explained.

Since the spectral analysis indicated that rhythmic LH patterns persisted during LH infusion, the mean period for these extremely rhythmic frequencies was examined for a possible effect of exogenous LH (Table I). When calculated on an equivalent basis (see footnote to Table I), there was no difference between control and experimental sequences. This fact would seem to minimize the possibility that the plasma LH fluctuations resulted from abrupt changes in the rate of removal of exogenous LH from plasma. It was shown previously (8) that the plasma LH fluctuations in untreated ovariectomized

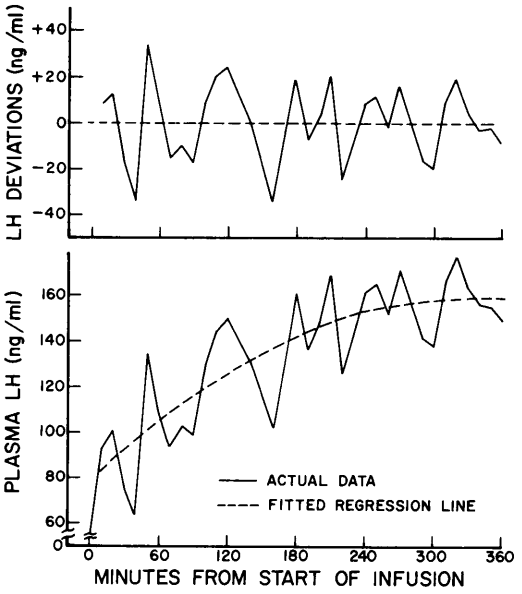

FIG. 1. Plasma LH pattern during one experimental sampling sequence. Lower graph depicts the actual concentrations in the sequential 10-min samples as well as the fitted curvilinear regression line. In the upper graph are plotted the deviations of the actual concentration at each 10-min interval from the regression line.

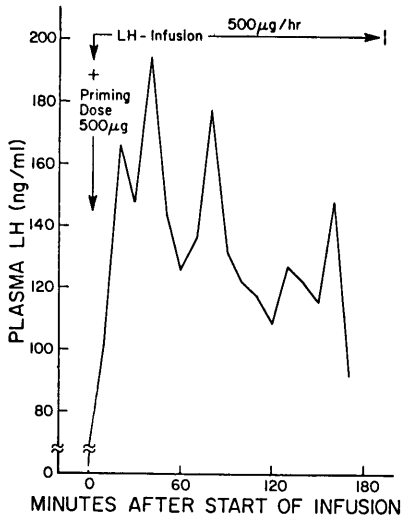

FIG. 2. Plasma LH pattern during a short (3 hr) trial in which a priming dose (500 μg) of exogenous LH preceded the constant infusion (500 μg/hr). The rate of constant infusion was apparently unable to fully maintain the elevated plasma LH concentration produced by the priming dose and LH levels declined during the second and third hours of the trial.

sheep resulted from pulsatile discharges of LH release from the pituitary rather than from rhythmic changes in LH turnover in plasma.

In all 14 trials summarized in Table I, exogenous LH was infused at a constant rate beginning at the start of the trial. Plasma LH began immediately to increase, but the rate of infusion exceeded the rate of removal from plasma for about the first hour during which time plasma LH levels continued upward. Figure 2 presents the results of a short (3 hr) experimental trial in which a priming dose of 500 μg LH was given in addition to the usual constant infusion. The priming dose abruptly increased plasma concentrations to a level that could not be sustained by the constant infusion. Despite the rapid increase, the plasma LH pattern in Fig. 2 reveals three sharp peaks of plasma LH which probably reflects the pulsatile release of endogenous LH. Therefore, the failure of infused LH to reduce plasma LH fluctuations or modify rhythmic patterns (Table I) cannot be ascribed to the gradual increase in circulating exogenous LH.

General discussion. The present results refute the hypothesis that elevated plasma LH resulting from each pulsatile discharge inhibited the next discharge until plasma LH had declined to low levels. However, there remains the possibility for a local pathway of short-loop feedback in which hypothalamic concentrations of LH might exceed the high plasma concentrations produced in the present experimental trials. The present results are similar to those reported in ovariectomized monkeys (13). In those studies the infusion of several preparations with the biological activity of LH did not affect the plasma pattern of endogenous LH. These workers were able to separately quantify endogenous LH because the infused preparations did not display immunoreactivity in their radioimmunoassay. In contrast, endogenous LH in the present study could not be directly quantified independent from the exogenous LH. However, the present approach assured that the animal would respond in the same manner to both exogenous and endogenous hormone.

The present inability to demonstrate the existence of inhibitory short-loop feedback of LH was consistent with similar observations for PRL and TSH feedback (3, 4). The only study involving short term elevations of circulating levels of AP hormone which inhibited endogenous release of that hormone involved GH (2). On the contrary, long term treatment with various AP hormones clearly inhibits in time the endogenous release of that hormone (1).

The present results raise the alternative possibility that the pulsatile release of endogenous LH may be greater during the infusions of exogenous LH. If elevated circulating LH does indeed increase the magnitude of the pulsatile discharges, it would be an example of the stimulatory type of short-loop feedback and not presently explainable in physiological terms.

Summary. Exogenous ovine LH was constantly infused during 6-hr experimental sequences in order to determine the effect of elevated levels of plasma LH on the pulsatile discharges of pituitary LH characteristic of ovariectomized sheep. Plasma LH concentrations (exogenous plus endogenous) in sequential 10-min samples continued to fluctuate significantly as did plasma LH (endogenous only) during control periods.

The temporal patterns of plasma LH during LH infusion were at least as rhythmic as they were in the controls. Furthermore, the mean periods for extremely rhythmic frequencies were equivalent in control and LH-infusion sequences. These data refute the original hypothesis that inhibitory short-loop feedback of circulating LH governs the timing of subsequent LH discharges in ovariectomized sheep.

Purified ovine LH was provided by the Hormone Distribution Officer, NIAMD, NIH and by Dr. L. E. Reichert, Emory University. The anti-ovine LH sera was provided by Dr. G. D. Niswender, Colorado State University.

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Received June 21, 1974. P.S.E.B.M. 1975, Vol. 148.