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Nonclassical Secretory Dynamics of LH Revealed by Hypothalamo–Hypophyseal Portal Sampling of Sheep

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Continuous withdrawal of hypophyseal portal blood from unrestrained sheep has permitted detailed assessments of the pulsatile secretion of gonadotrophin-releasing hormone (GnRH). To determine if this blood can also be used to characterize the secretory dynamics of pituitary hormones, patterns of luteinizing hormone (LH) in the hypophyseal portal blood of ovariectomized ewes was compared with previous patterns of GnRH and peripheral LH. Hypophyseal portal blood and jugular vein blood were collected every 5 min from six ovariectomized ewes over 6–12 h. Hypophyseal portal blood contained GnRH-associated, sharply defined LH pulses that were much larger than in the periphery. Pulses of secreted LH (hypophyseal portal LH less peripheral LH) showed much faster rates of rise and fall than peripheral and followed pulses of GnRH by an average of 1.26 min. In contrast to pulses in jugular blood, secreted LH pulses often reached a relatively unchanging interpulse nadir–plateau and thereby approached closely algorithm-estimated, extrapolated baselines. The interpulse baseline concentrations of secreted LH (99.6 ng/mL) in hypophyseal portal blood were 31-fold higher than those for jugular LH (3.23 ng/mL). These elevated concentrations also exceeded mean jugular peak concentrations (11.1 ng/mL) and, thus, primarily must represent newly secreted LH. The non-Gaussian profiles of this secreted LH were substantially more complex than the inputs predicted from jugular LH measurements by deconvolution. Furthermore, regardless of the analytical approach, estimations of the mass of secreted LH in each pulse did not correlate well with inputs predicted by deconvolution or Kushler–Brown pulsefit analysis of corresponding pulses in jugular blood (r^2 ranging 0.40–0.48). Among alternative explanations is the possibility of heterogeneity in concentrations of GnRH in the portal vessels and variable distribution within the hypophysis. In summary, assay of hypophyseal portal blood obtained directly from the pituitary provides a method for direct assessment of secretory responses to hypothalamic peptides, and thereby serves as an unmatched method for studying the dynamics of LH secretion in vivo. With this approach, LH is revealed to be secreted as complex, non-Gaussian pulses that are far more sharply defined than those in the periphery, include non-GnRH-dependent, secretory components that cannot be predicted by deconvolution and are followed by periods of relatively constant, basal secretion.

Key Words: Gonadotrophins; reproduction; GnRH; luteinizing hormone; deconvolution.

Introduction

Luteinizing hormone (LH) is secreted episodically by gonadotropes in the anterior pituitary gland and appears in peripheral plasma as pulses *(1–6)*. Identification and characterization of these pulses have been approached by a variety of statistical and modeling techniques *(7–14)* with the expectation and subsequent demonstration that gender-, age-, physiological state-, treatment-, and disease-specific changes in the frequency or amplitude of the measured LH pulses would provide insights into the underlying changes in hypothalamic-derived gonadotrophin-releasing hormone (GnRH). Validation of this premise was provided when methods capable of measuring GnRH sequentially in the hypothalamic–hypophyseal portal blood revealed a one-to-one correspondence between the peripheral pulses of LH and far sharper and discrete pulses of GnRH in the hypophyseal portal blood *(15–17)*.

Hormone secreted by the anterior pituitary gland is subjected to dilution, dispersion, mixing, and elimination. The convolution of these processes creates the profiles measured in the peripheral blood. Relative to typical sampling times of 5–20 min and circulatory times of nearly 1 min *(18)*, the dominant process acting on the secreted product is elimination. With assumptions of unchanging single or multiexponential elimination, or of a fixed secretory wave-

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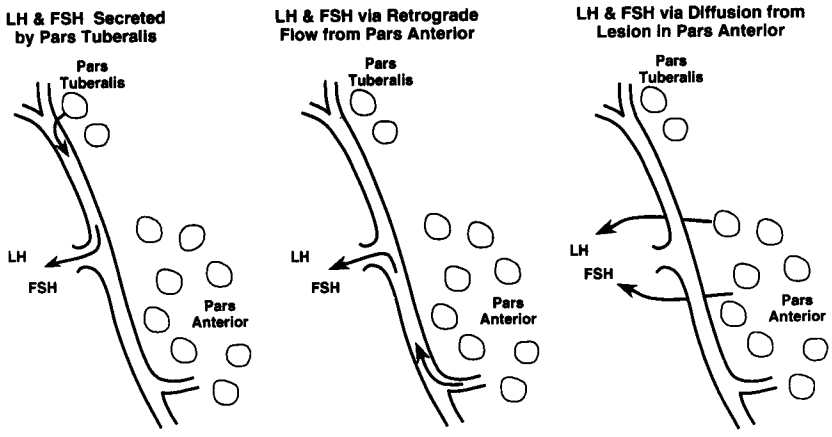

Fig. 1. Three possible modes by which secreted LH might reach the collected hypothalamo–hypophyseal portal plasma: secretion originating in cells of the pars tuberalis, retrograde flow, and leakage from lesioned sinusoids within the body of the gland.

form, peripheral concentrations have been deconvolved to obtain a better estimate of the secretory dynamics of LH *(6,12,19)*. These methods, derived in part from pharmacokinetics, have been applied to characterize the secretory bursts of pituitary LH, their half duration, interburst intervals, secreted mass, and secretion rates. Additionally, the data have provided estimates of elimination half-life. The deconvolution may have multiple solutions and the actual character of the secretory inputs, generally modeled as Gaussian waves, are not known. They could be far more complicated. Needed are ways to assess the secretory events more directly.

Continuous withdrawal of hypophyseal portal blood, a method pioneered by Clarke and Cummins *(15)* and refined by Caraty and Locatelli *(16)*, has provided a direct and detailed understanding of the pulsatile secretion of GnRH in sheep. This method involves surgical insertion of a blood collection device through the sphenoid bone just below the hypothalamus and terminating at the face of the pituitary. Collection of hypophyseal portal blood is accomplished by lesioning portal vessels on the anterior pituitary. Because the collection site is close to the site of release of GnRH, GnRH is effectively not detectable in peripheral circulation, and GnRH is measured during its single pass down the portal vessels, the method has provided a close approximation of the kinetics of GnRH secretory patterns *(20)*.

Recognizing that the cuts made for collection of hypophyseal portal blood at the anterior pituitary also involve lesioning vascular sinusoidal vessels, it was reasoned that the collected blood not only provides a means to assess patterns of secreted GnRH, but might also serve as a means to assess with precision the dynamics by which hormones are secreted by the pituitary. As illustrated in Fig. 1, LH might enter the portal circulation via three routes that are not mutually exclusive:

1. From active secretion of gonadotropes located in the pars tuberalis surrounding the stalk of the adenohypophysis *(21)*;
2. From retrograde flow of vessels distal to the lesion either from retrograde flow in the stalk *(22)*, or from a shift in pressures secondary to lesioning; and
3. From drainage of sinusoids lesioned in the pituitary.

If LH were to enter portal blood in any of these ways, it was postulated that measuring LH in the portal circulation would provide a truer picture of secretory events than would measurements in the peripheral circulation. In this report for the first time evidence is provided that the same methods used to generate profiles of episodic GnRH activity in a time series of hypophyseal portal blood, also can be used to obtain profiles of secreted LH, and that these profiles can be characterized as rapidly changing, non-Gaussian, short bursts of secretion that are interspersed by far longer periods of basal secretion.

Results

A representative pattern of secreted LH in an ovariectomized ewe sampled during the anestrous season with concentrations displayed on an arithmetic axis is shown in Fig. 2A. For comparison, the previously reported pattern of jugular LH in this ewe is also presented. Concentrations of secreted LH in the hypophyseal portal plasma can be one to two orders of magnitude higher than in the jugular plasma. Each LH pulse in the peripheral plasma coincides with a corresponding pulse of secreted LH in the hypophyseal portal plasma. Furthermore, pulses of secreted LH in the hypophyseal portal plasma are far more discrete than pulses in the peripheral jugular plasma. A logarithmic plot of the same data, as illustrated in Fig. 2B, facilitates recognition of these comparisons, expands changes in lower baseline concentrations, and reduces heteroscedasticity. Consequently, the remaining figures are displayed with this transform.

Figure 3 summarizes the patterns of hypophyseal portal LH from an ovariectomized ewe sampled during the breeding season. Patterns of hypophyseal portal GnRH and jugular LH (J-LH) *(23)* are coplotted for comparison with conversion of GnRH values from amounts secreted per minute to concentrations. As documented earlier *(15–17,23)*, GnRH is released in discrete episodes returning to undetectable or nearly undetectable baseline concentra-

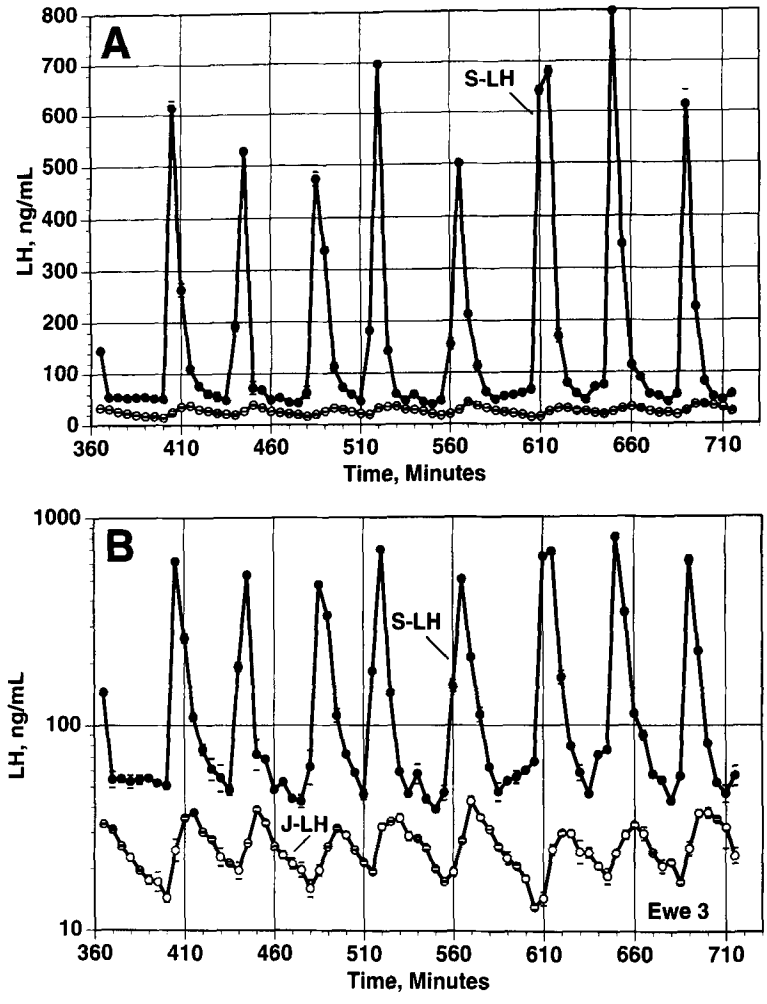

Fig. 2. Patterns of hypophyseal portal LH and jugular LH from an ovariectomized ewe during the anestrous season. Concentrations of jugular LH (J-LH) are from a previously published study *(23)* and are coplotted for comparison. **(A)** The hormone concentrations are plotted on an arithmetic axis to provide a sense of the magnitude of differences in the levels of LH present in the portal and peripheral circulation. Hypophyseal portal LH concentrations, with the indicated jugular concentrations subtracted, are plotted as secreted LH (S-LH). **(B)** The same data displayed in a logarithmic transform to reduce nonuniformity of variance and reveal more clearly changes in the lower concentrations.

tions. Each such episodic pulse of GnRH is directly associated with a pronounced episodic burst of secreted LH in hypophyseal portal plasma and a succeeding, far broader pulse of LH in jugular plasma. As would be expected from continued elimination and bursts of secretion, the nadirs of jugular LH appear to be lowest when the interpulse interval is longer. In no case in this study did the concentration of LH in jugular plasma reach the baseline concentration predicted by the Kushler–Brown pulsefit algorithm *(14)*. In contrast to changes in peripheral LH, the response of secreted LH to the onset of an episodic GnRH burst appears to be very rapid, often rising within the same sampling interval of 5 min for both hormones. Also, in contrast to changes in peripheral LH, the subsequent fall in secreted LH is rapid, but appears to be slower than that for GnRH. In addition, as best seen in Fig. 2A, the pulses of secreted LH return to a sustained, but measurable quasi-plateau concentrations that occasionally appear to rise between pulses (e.g., 585–605 min).

Similar profiles, shown in reduced format for part (ewe number 5) or all of the samples from the other four ovariectomized ewes, are shown in Fig. 4. Three of these ewes were sampled during the breeding season and one (number 5, like number 3, Fig. 2) during the nonbreeding season. The GnRH, hypophyseal portal, and jugular LH relationships, detailed for Fig. 3, also pertain to Fig. 4. Data from all four ewes are graphed to the same scale to permit direct comparisons. Note, in particular, the variability in magnitude of the pulses of secreted LH, both within a series for one ewe and between series from different ewes.

The characteristics of LH secretory episodes from each ewe, as determined by the Kushler–Brown pulsefit algorithm *(14)* and by deconvolution are described in Table 1. Shown for each ewe are the sampling duration, number of pulses identified during this period, pulses/h, baseline concentrations, pulse amplitude, and half-time disappearance

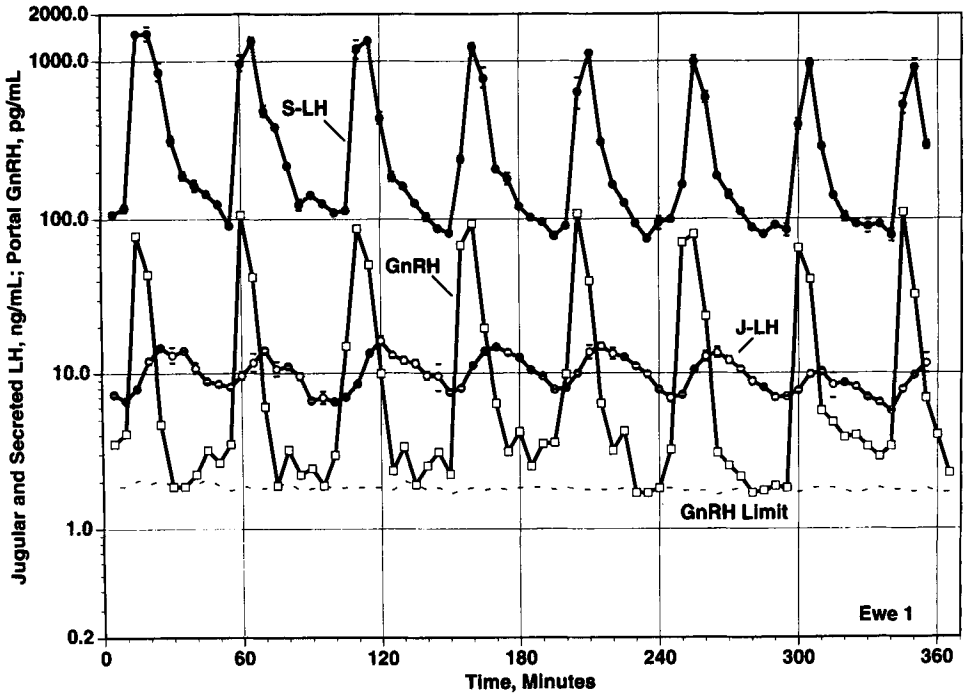

Fig. 3. Hypophyseal portal GnRH, secreted LH, and jugular LH patterns from an ovariectomized ewe during the breeding season. GnRH and jugular LH (J-LH) concentrations are from a previously published study *(23)* with the GnRH converted from amounts per min to concentrations and coplotted for comparison. The hormone concentrations are plotted on a logarithmic vertical axis versus arithmetic time along the horizontal axis. Hypophyseal portal LH concentrations, with the indicated jugular concentrations subtracted, are plotted as secreted LH (S-LH). The line at the bottom, designated GnRH Limit, represents the described tube-specific concentration limit of detection in this assay. The irregularity of the line reflects the variable volume of sample actually assayed in each tube as a consequence of variable amounts of blood being delivered into the fixed volume of bacitracin. The small horizontal bars on either side of the plotted concentrations of jugular and secreted LH (but not GnRH) represent the range of duplicate values. When the bars cannot be discerned, they are within the size of the symbol.

of the identified GnRH and LH pulses. To achieve homogeneity of variance, means and standard errors of the mean were calculated after logarithmically transforming all values; the table displays the converted antilogarithms. All hormonal series fit the models assumed both by the Kushler–Brown pulsefit algorithm and by deconvolution.

As is evident from the table, the number of LH pulses identified by the Pulsefit method in the hypophyseal portal plasma (and to a lesser extent by deconvolution) were virtually identical to the number of LH pulses identified in the periphery and to the number of GnRH pulses identified in the hypophyseal portal plasma with differences deriving from constraints of pulse detection criteria at the ends of the series. The average number of pulses per hour for all three variables ranged from 1.39–1.41 (a pulse every 43 min). As reported for the larger group *(23)*, basal GnRH concentrations were at or near the limit of detection and here averaged 1.12 pg/mL. The mean, model-estimated concentration of baseline LH (99.6 ng/mL) was 31-fold greater than that estimated for peripheral LH (3.23 ng/mL). Similarly, mean pulse amplitude of secreted LH was 54-fold greater than the amplitude of peripheral LH pulses. As reported previously, the half-time of GnRH disappearance was very rapid, here averaging 1.67 min. The disappearance time of LH in the hypophyseal portal plasma, although not as fast as GnRH, was much faster than that seen for LH in the periphery (with half-time of disappearance of secreted and peripheral LH estimated to be 4.35 and 24.2 min, respectively). Table 1 reveals similar results by deconvolution; observed differences in estimated disappearance half-time may result from sampling only once every 5 min.

The average lag between the times of pulse onset, as identified by the pulsefit algorithm, was determined by auto cross-correlation analysis for GnRH and portal LH, portal LH and jugular LH, and GnRH and jugular LH. To enhance the pulsatile component of the temporal relationships, only those points that were on or near the GnRH pulses were used. The results are shown in Table 2. With exception of one concordant pulse, pulses of GnRH started slightly before pulses of LH in portal plasma by an average of 1.3 min. The portal pulses of LH appear to precede the pulses in the jugular plasma by a little over 2 min.

To learn how closely these direct measurements compare with events predicted by deconvolution of peripheral concentrations, all data was analyzed using our implementation (MB) of the method of deconvolution *(6,12,19)* and compared deconvolved input predictions derived from LH in jugular blood to values observed in hypophyseal-portal blood. A typical outcome for the first 6 h of collection from

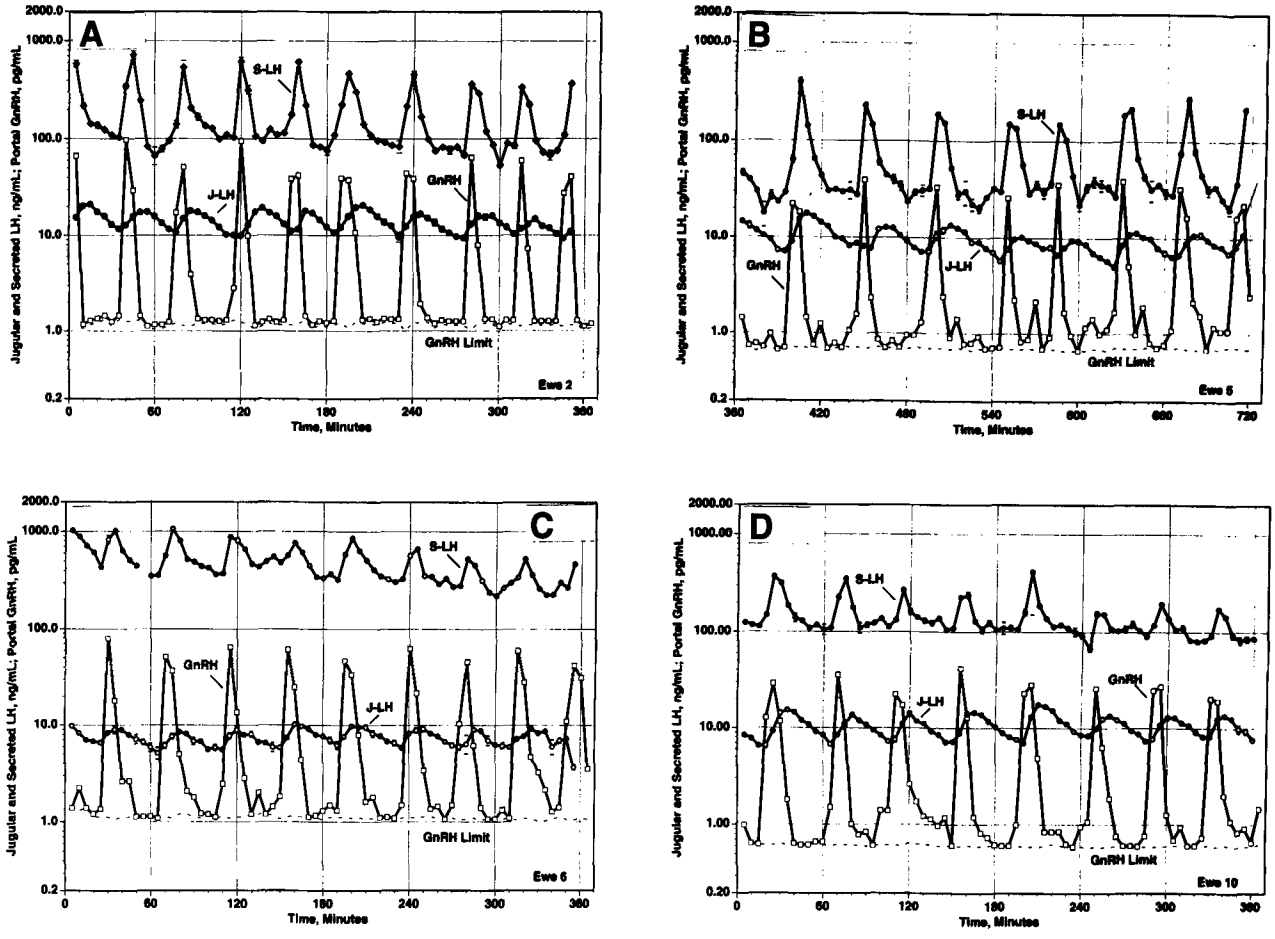

Fig. 4. Results of concentrations of hypophyseal portal GnRH, secreted LH, and jugular LH for four ovariectomized ewes. Three of the ewes (numbers 2, 6, and 10) were sampled during the breeding season while one (number 5) was sampled during the nonbreeding season. Results for all ewes are plotted on axes of the same scale and dimension to facilitate comparison. (See the caption to Fig. 3 for an explanation of the symbols.)

ewe number 3 is shown in Fig. 5. The deconvolved profiles, typically assumed to be Gaussian as shown here, are represented by the dashed lines. Even with the large sampling interval of 5 min, striking discrepancies can be observed. In contrast with the deconvolved profiles, the duration of the observed secretory events at half-maximal amplitude are shorter, the period of maximal secretion appears to be followed by a discontinuity suggestive of a possible secondary secretory addition, and this phase is followed by a period of continued, basal secretion not reflected in the deconvolution.

By visual inspection, the mass of secreted LH and the size of the pulses in the jugular circulation do not always seem to bear simple one-to-one relationships. For analysis of these paired pulse-size relationships, deconvolution *(6,12,19)* and the Kushler–Brown pulsefit algorithm *(14)* were used to estimate separately the amount of LH input for each portal and jugular pulse. For this, the Kushler–Brown estimates were calculated after subtracting pulse-specific baselines determined as the mean of three values prior to the first upswing. These pulse input estimates (portal and jugular) were used either as calculated or after log-transformation and then, to permit interanimal comparisons, normalized by dividing by the mean of values for pulses for each ewe. These normalized, paired estimates of input (portal vs jugular) were then subjected to regression analysis. Regardless of transform, the correlation coefficients for these paired estimates of input were low, ranging 0.40–0.48. Similar correlation coefficients were observed for individual ewes with mean correlation coefficients of 0.44 (deconvolution) and 0.43 (pulsefit) with extremes of 0.012 and 0.82. Surprisingly, correlation coefficients for similar comparisons between deconvolved pulses of GnRH and portal LH ($r^2 = 0.29$) and GnRH and jugular LH ($r^2 = 0.10$) were even lower.

Discussion

Development of surgical approaches to obtain hypophyseal portal blood from conscious animals has provided us with a means to assess changes in secretory dynamics of GnRH *(15–17)*. In this study, it is demonstrated that this very same approach can also be utilized to obtain unprecedented and rich insights into the in vivo secretory dynam-

Table 1
Summary of Pulse Analysis Using the Kushler–Brown Algorithm *(14)* and Deconvolution *(6,12,19)*

GnRH			Kushler–Brown†				Deconvolution†	
	No. h	No. peaks	No. pulses per h	Baseline pg/mL	Amplitude Δ pg/mL	Disappearance half-time, min	Amplitude Δ pg/mL	Disappearance half-time, min
Ewe 1	6	8	1.33	2.59	87.4	2.19	97.3	2.91
Ewe 2	6	9	1.50	1.17	54.1	1.30	65.6	1.97
Ewe 3	12	16	1.33	0.79	60.9	1.45	63.0	2.27
Ewe 5	12	16	1.33	0.88	36.2	1.19	33.7	3.10
Ewe 6	6	9	1.50	1.27	54.6	2.22	65.6	2.53
Ewe 10	6	8	1.33	0.73	27.4	2.01	36.4	2.32
Mean (±SEM)*			1.39	1.12	50.0	1.67	56.4	2.49
			0.04	(+0.23, –0.19)	(+9.0, –7.7)	(+0.20, –0.18)	(+10.1, –8.6)	(+0.18, –0.16)
Secreted LH			No. pulses	Baseline	Amplitude	Disappearance	Amplitude	Disappearance
	No. h	No. peaks	per h	ng/mL	Δ ng/mL	half-time, min	Δ ng/mL	half-time, min
Ewe 1	6	8	1.33	98.4	1064	3.90	1300	4.18
Ewe 2	6	9	1.50	102.4	391	2.94	446	5.35
Ewe 3	12	16	1.33	75.8	735	3.11	974	3.36
Ewe 5	12	16(19)	1.33	45.6	290	4.29	323	5.52
Ewe 6	6	10(12)	1.67	259.0	437	9.07	528	6.71
Ewe 10	6	8	1.33	108.5	147	4.89	205	7.96
Mean ±SEM*			1.41	99.6	423	4.35	520	5.30
			0.06	(+25.9, –20.6)	(+139, –105)	(+0.79, –0.67)	(+168, –127)	(+0.72, –0.63)
Jugular LH			No. pulses	Baseline	Amplitude	Disappearance	Amplitude	Disappearance
	No. h	No. peaks	per h	ng/mL	Δ ng/mL	half-time, min	Δ ng/mL	half-time, min
Ewe 1	6	8	1.33	3.51	6.3	24.0	4.3	12.4
Ewe 2	6	10(11)	1.67	4.87	8.4	22.0	3.9	8.6
Ewe 3	12	16	1.33	2.97	21.8	25.8	15.6	12.1
Ewe 5	12	15(17)	1.25	1.66	7.2	30.5	5.5	12.8
Ewe 6	6	9(8)	1.50	3.02	3.4	26.8	2.8	18.2
Ewe 10	6	8	1.33	4.50	8.5	18.2	4.5	11.8
Mean ±SEM*			1.40	3.23	7.9	24.2	5.1	12.3
			0.06	(+0.55, –0.47)	(+2.2, –1.7)	(+1.8, –1.7)	(+1.4, –1.1)	(+1.3, –1.1)

*The nonequal values for the SEMs of the estimates of baseline LH, amplitude, and disappearance half-time result from calculating the means and standard errors as logarithmic transforms and then presenting the antilogarithms here.

†With the deconvolution method, amplitude is the total input; for Kushler–Brown, it is the trough to peak difference. With exception of identification of interpulse variation as a few additional "peaks" (in parentheses), deconvolution gave the same number of peaks as Kushler–Brown.

Table 2
Average Pulse Lag Times in Min (Kushler–Brown *[14]*)

Sheep	No. common pulses	GnRH-portal LH	GnRH-jugular LH	Portal LH-jugular LH
Ewe 1	8	1.25	3.12	1.88
Ewe 2	9	0.56	3.33	2.78
Ewe 3	16	0.00	3.30	2.50
Ewe 5	16	2.81	4.67	2.00
Ewe 6	9	1.67	1.88	0.62
Ewe 10	8	1.25	3.75	2.50
Mean ±SEM		1.26 ± 0.43	3.34 ± 0.41	2.05 ± 0.35

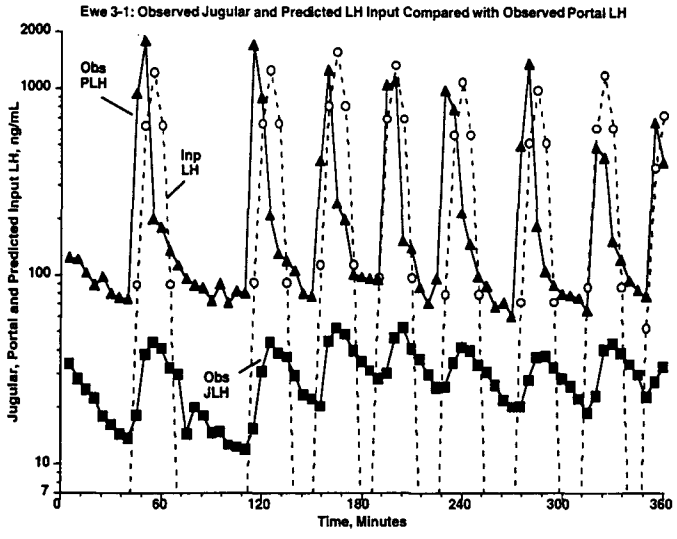

Fig. 5. Measured jugular LH (JLH) and portal LH (PLH) compared with LH input as predicted by deconvolution of jugular results (Inp LH; dashed line) for the first 6 h of sampling of ewe number 3.

ics of at least one gonadotrophin and presumably other pituitary hormones.

Utilizing this unique approach, it was found that LH is secreted in discrete, short, episodic bursts with relatively rapid termination times. More importantly, termination of the active phase of secretion is not followed by continued decline of LH. Instead, LH often appears to reflect secondary secretory events and then to remain at low, but clearly substantial levels during interpulse periods. This is consistent with the occurrence of continued low, but steady secretion. Since all of the measurements of LH were read in a region of high precision and none approached the limits of detection, it would appear that LH is secreted in at least two modes: a GnRH-stimulated, episodic mode and a more continuous, steady mode that is separate from pulse-related release. Whether or not this continuous, basal secretion of LH is also under the control of GnRH, regulated by feedback, e.g., by ovarian products like estradiol or by other hypothalamo-hypophyseotropic agents in the portal blood, remains to be determined. Also, whether or not secondary, lesser secretory events are part of some pulses will need to be determined by more frequent sampling.

The profiles of secreted LH reported here cannot be exact replicas of secretory events at the gonadotrope since they are the product of convolutional events within the pituitary gland. It is likely, however, that they represent the closest approximation available to date of secretory dynamics from at least a portion of the pituitary gland.

Prior understanding of secretory aspects of pituitary hormones in vivo has come primarily from deconvolution of the patterns derived from peripheral measurements of hormone that has been subjected to dilution, dispersion, and clearance. Approaches to measure pituitary secretory dynamics directly have been limited. Direct characterization of pituitary secretion is increasingly becoming possible because of the development of methods that allow sampling of blood at a site close to the pituitary. In particular this has been accomplished by sampling of intracavernous sinus blood in sheep *(24)* and horses *(25–29)* and imaging-monitored catheterization and sampling of the inferior petrosal sinus in humans *(30)*. The intracavernous sinus approach has been successfully utilized in the horse to define the secretory pattern of gonadotrophins and its relationship to GnRH *(25)*. In contrast, the inferior petrosal sampling that is developed in the humans to lateralize the site of a pituitary adenoma, has proven to be less useful in characterizing gonadotrophin secretory dynamics because:

1. The blood collected at this site is mixed with contributions from a relatively large number of brain sites;
2. The diagnostic utility of the procedure limits long-term collection; and
3. The inability to measure GnRH in these samples limits the utility of the approach to study GnRH-LH interrelationships.

In spite of such caveats, these studies have revealed relatively higher concentrations of pituitary LH and follicle-stimulating hormone (FSH) in the petrosal sinus than in the periphery *(31)*, although not of the magnitude seen in horses *(25–29)* and sheep (this study).

A major advantage of the hypophyseal portal approach for monitoring pituitary secretory dynamics as compared with the approaches described in horses and humans relate to the resolution of the secretory signal achieved. Because portal vessels are cut at the surface and within the anterior pituitary, collection of hypophyseal portal blood involves direct access to pituitary sinusoids and the pituitary secretory products before they are subjected to dilution during transit to the cavernous sinus. Subsequently, collection of air-segmented portal blood preserves the delivered signal during transit in the collection tubing. This approach also provides a means to monitor the patterns of LH and GnRH secretion simultaneously.

As has been reported for pulses of jugular LH and hypophyseal portal GnRH *(17)*, pulses of hypophyseal portal LH in ovariectomized ewes occur at a rate of approx 1.4 per h and exhibit a nearly perfect concordance with pulses of GnRH and jugular LH. In comparison with LH, pulses in the periphery (disappearance half-time of approx 25 min), hypophyseal portal LH pulses are more discrete with a mean disappearance half-time of approx 4.3 min. Studies using pituitary venous blood of mares during the midluteal phase have revealed pulses that are infrequent (eight pulses per 80 h) and prolonged with secretion of GnRH and LH each lasting for 30–55 min *(26)*. However, following prostaglandin-induced luteal regression, the number of LH pulses increased *(29)* and the resulting pulses were similar to those reported here with rapid rates of increase, the bulk of the pulse contained in a single sample, and a slower decline to reach a plateau phase of continuous secretion. Thus,

whereas the reproductive endocrinology of LH secretion in the horse has a number of unique features, it also shares a number of similarities with LH in sheep. Unfortunately, since simultaneous measurements of GnRH in the pituitary venous blood or LH in the peripheral blood were not reported in this latter study *(29)*, parallels in the magnitude of changes of LH between peripheral and secreted LH cannot be drawn.

Interestingly, the characteristics of LH secretory episodes reported here are remarkably close to those calculated 24 yr ago by Butler et al. *(4)* who studied the secretion of LH in ovariectomized ewes by measuring the arteriovenous differences in concentration of LH in nearly simultaneous arterial and jugular vein samples obtained at 15 min intervals. The current results also support work by Rasmussen and Malven *(32)* who sampled arterial and jugular vein blood across the head every 20 s. Not only did their studies reveal the pulsatile character of ovine LH and show that the pulses resulted from secretion and not changes in metabolism, they also led to an estimate that each pulse of LH only needed to last 5 min to account for the observed changes in peripheral concentrations. Butler et al. *(4)* also estimated disappearance rates for two pulses as 22.5 and 26.5 min. The estimates and projection of secretory episodes reported by Butler et al. *(4)* conducted shortly after the pulsatile character of LH had been revealed in rats *(1)* and monkeys *(3)*, and even before GnRH had been isolated, prove to be correct based on our results.

Most of the LH in hypophyseal portal plasma must represent active secretion, and the slower disappearance of LH vs GnRH likely results from secretion that continues after the GnRH signal has disappeared. These conclusions appear probable for three reasons:

1. The concentrations of LH in portal circulation are far too high to be accounted for by recirculating LH;
2. The small volumes of blood leaving the pituitary are diluted by a relatively huge circulation such that the concentrations in portal blood effectively, but not exclusively, represent one time passage; and
3. Since elimination is convolved with dispersion, disappearance of GnRH must represent close to the maximum time for any substance to be carried through the portal circulation.

Indeed, the difference in size (≈28,000 Daltons for LH; ≈1000 Daltons for GnRH) should lead to greater diffusivity of GnRH and, therefore, greater dispersion and slower washout. If LH secretion were to terminate as rapidly as GnRH, it should be washed through at least as quickly. Overall, the half-time for disappearance of LH from portal plasma was 2.6 (pulsefit) to 2.1 (deconvolution) times longer than that for GnRH.

For these reasons, concentrations of LH in jugular plasma were subtracted from concentrations in portal plasma and designated the difference as "secreted" LH. Since these samples were obtained directly at the pituitary and since rates of change in hormone concentrations in these samples were far more rapid than those in the periphery, these concentrations can be taken as representing a close approximation to the actual secretory dynamics of gonadotropes releasing LH into sinusoids of the portion of the pituitary that has been lesioned. This conclusion must be tempered, however, since it is likely that some of the LH secreted in other regions of the gland arrived at the surface by circuitous routes and this might account for some of the delay in decline of LH over GnRH.

Remaining to be seen is whether or not these profiles are representative of secretion in the remainder of the pituitary. Since the blood removed is not returned to the body, LH measured in the hypophyseal portal plasma cannot contribute to the concentrations observed in the jugular vein. Further, since the sampled hypophyseal portal plasma represents an unknown fraction of the total input, it is not possible to use the concentrations of LH in the hypophyseal portal plasma to calculate total secretion. To the extent that the observed profiles are representative, however, they can be used as guides providing an insight into the dynamics of pituitary LH secretion. Since the size and dynamic profiles of peripheral pulses do not appear to be substantially different between intact and portal-sampled sheep *(17)*, it would appear that only a small amount of LH is removed in the hypophyseal portal blood and that the sampling procedure is not overly disruptive of pituitary secretion.

The observed poor correlation between mass of secreted LH predicted from pulses of jugular LH and pulses of portal LH was unexpected. The rate of flow of portal blood does vary some with the position of the head. The possibility thus exists that these differences might result from differences in relative flow rates between hypothalamic-hypophyseal portal circulation and sinusoidal flow in the pituitary. Examination of the volumes of portal blood collected in each sample, however, failed to reveal this as a source of the extremes in differences between sizes of pulses of portal and jugular LH. The results do provide support for the possibility that hypothalamic signals are distributed differentially among the portal vessels with the contents of lesioned vessels not being representative of what is being delivered to other portions of the pituitary, portions that are responsible for delivering the LH that reaches the peripheral circulation. Clearly, gonadotropes and other cell types are distributed differentially within the pituitary *(21,33)*, and the portal vasculature is highly complex *(22)*. Another explanation might be that differences result from local, intrapituitary changes in distribution of blood flow.

A related, but conceptually different issue, is the surprisingly poor relationship between the mass of measured GnRH and the resulting response of the pituitary as reflected by secreted and peripheral LH. Part of the difference might result from exceeding maximally effective concentrations of GnRH. Whereas some part of these

poor correlations may result from differences in vascular flow, as mentioned, the results could also result from inclusion within the portal circulation of hypothalamic factors affecting LH secretion other than GnRH.

Whereas the rates of rise and fall of GnRH appear to be fairly similar, the shapes of the secreted LH pulses appear to be skewed with a disappearance that is slower than the rise time, i.e., non-Gaussian. To assess these apparent differences with more care and to obtain a more detailed understanding of the dynamics of secretion, sampling will need to be done at a substantially faster frequency. With the 5 min sampling interval used here, the time from baseline to peak was less than the sampling interval for many of the pulses. In spite of these limitations, measurement of LH in portal samples appears to provide a substantially better approximation of LH secretion than can ever be obtained by deconvolution as revealed in Fig. 5 and Table 1 *(6,9,10)*.

Hormone concentrations in Figs. 2–4 are plotted on a logarithmic axis that accentuates changes in low concentrations. Nevertheless, an examination of the figures provides some evidence to suggest that minor excursions in GnRH during interpulse intervals are related to corresponding minor interpulse increments in LH, e.g., ewe number 5 at 385, 570, 610, and 685 min. Although suggestive of a cause and effect relationship, this association is not consistent and the relevant concentrations of GnRH, 1-3 pg/mL, are at the lower limits of what are generally reported to be the physiological dose-response range for GnRH with sheep gonadotropes. Thus, these observations must be treated with caution.

Attention should also be drawn to what appears to be a gradual decline in secreted LH over time in several of the ewes (numbers 1, 2, 6, and 10). This occurred in the absence of comparable changes in jugular LH or hypophyseal portal GnRH. Since jugular LH represents the convolved elimination and secretion from the rest of the pituitary, and since compensatory changes in elimination are not likely, it would appear that what is designated secreted LH is not fully representative of what is being secreted elsewhere. Why the decline would occur in the face of constant GnRH input is not clear. One possibility that might account for the decline is reduced local secretion in the region of the lesion, perhaps in response to an inflammatory response. The latter is made a little less likely in light of the results in ewes 3 and 5, each of whom were studied for 12 h and failed to show substantial decline over this duration. It should be noted that the trend downward in LH concentrations in some ewes was not accompanied by a concomitant change in the volume of blood draining from the pituitary into our collection apparatus.

In summary, hypophyseal portal blood, usually collected for measurement of hypothalamo-hypophyseotropic agents such as GnRH, contains high concentrations of LH. The LH largely represents nascent, first-passage secretion from gonadotropes in the pituitary gland. Time-dependent profiles of LH in this blood from ovariectomized ewes reveal sharply defined, episodic, brief, non-Gaussian bursts with faster rise than decline phases that are interspersed with longer periods of relatively quiescent, low concentration, continued secretion. The bursts appear within 1–2 min following an even more sharply defined pulse of GnRH and are followed a few minutes later by far broader pulses that have been well-defined in the peripheral circulation. Importantly, the profiles reveal complexity and the existence of non-GnRH-dependent, secretory components that cannot be predicted by deconvolution. This is the first demonstration of the relationship in vivo between GnRH and the dynamics of LH secretion, a relationship that should provide important mechanistic implications and insights into the nature of fast acting desensitization. Through studying these relationships, the potential exists to achieve a rich understanding of the regulation of LH secretion and likely other pituitary hormones such as FSH in vivo.

Materials and Methods

Experimental Design

For characterization of secretory patterns of LH in hypophyseal portal blood of ovariectomized ewes, the authors capitalized on samples obtained from heparinized sheep in a previous study *(23)*. Thus, detailed description of the surgical procedures, sample collection, and patterns of GnRH in hypophyseal portal blood and LH in jugular blood have been described previously *(23)*. In brief, adult Suffolk ewes, maintained at a Sheep Research Core facility were ovariectomized and surgically fitted with an apparatus for collection of hypophyseal portal blood *(34)*. Approximately 1 wk later, collection of hypophyseal portal blood was initiated by lesioning the portal vessels at the anterior surface of the pituitary gland *(34)*. Integrated 5 min samples of hypophyseal portal blood and jugular blood were collected in tubes containing 0.5 mL of 3 m*M* bacitracin in phosphate-buffered isotonic saline with the tubes kept in ice water during collection. Plasma was separated by centrifugation and rapidly frozen by immersion in a bath of ethanol and dry ice. Samples collected for 6 h from four ovariectomized ewes (numbers 1, 2, 6, and 10) during the breeding season and for 12 h from two ovariectomized ewes (numbers 3 and 5) during anestrus, were utilized for this follow-up study. Plasma LH concentrations were measured in each of the hypophyseal portal blood samples and each series of portal LH measurements was compared with corresponding measurements of previously reported portal GnRH and jugular LH *(23)*.

All procedures were done with the approval of the University's Committee on Use and Care of Animals (UCUCA) and met guidelines promulgated by the NIH.

Immunoassays

Portal LH was assayed in duplicate by radioimmunoassay using purified ovine LH (AFP-7071B; NIDDK-oLH-I-

12) for iodination and polyclonal antiserum to oLH in accord with procedures described previously (35–38). An ovine serum-based standard, B1371, calibrated against NIH-LH-S12, was used as reference. The entire series of hypophyseal portal samples from each animal were measured together in a single assay. Values reading at <20% of the zero dose, were reassayed using a lower volume of serum. Except for reassay of such samples, 5 μL of hypophyseal portal plasma were used in each assay tube. The limit of detection of the six primary assays for the hypophyseal portal samples (two standard deviations from the zero control) averaged 89 pg/tube (range: 79–129). Two serum controls, run in each assay, had mean concentrations of 2.3 and 27.4 ng/mL. Intra-assay coefficients of variation of these controls averaged 5.2% and interassay coefficients of variation averaged 9.2%. The limits of detection and the intra- and interassay coefficients of variation for the subset of assays that have been utilized from the original study *(23)* for comparison with the hypophyseal portal LH patterns reported here averaged 53.0 pg/tube, 6.2%, and 11.4%, respectively for LH and 0.19 pg/tube, 16.7%, and 24.0% for GnRH. Retesting of some jugular samples with the same competitive, single site radioimmunoassay did not reveal significant differences and quality control pools have shown no loss of LH immunoactivity over time.

Adjustment of Sample Timing

To minimize dispersion, the collection rate was set at a higher speed than the flow rate such that the hypophyseal portal blood was collected as discrete blocks segmented by air *(34)*. This segmentation effectively eliminates dispersion in the tubing. The jugular blood was collected continuously and was not segmented by air. Thus, the jugular blood is subjected to substantial dispersion in the line leading from the sheep to the fraction collector, but this is of little consequence because of the slower rates of change in the peripheral circulation. Because the pumps were run at different speeds, the transit time for jugular blood varied from 10–12 min whereas that for the hypophyseal portal blood and air was closer to 3 min. After adjusting for this 7–9 min difference, the concentration of LH in each jugular sample was subtracted from the determined concentration in the simultaneously collected hypophyseal portal sample and the resulting difference designated as secreted LH. It should be noted, however, that differences in sampling interval leave a small error of 1–3 min and blood will disperse differently in the two lines (blood is removed continuously from the jugular but is interspersed with air in the line from the pituitary).

Calculation of Hormone Concentrations

To account for differences in blood volume collected during each sample collection period and possible contamination of hypophyseal portal blood by fluids from other sources (cerebrospinal fluid, peripheral blood), prior studies from this laboratory have reported results in portal plasma as amount of GnRH collected per minute *(17,23)*. To allow direct comparisons with previously reported concentrations of LH in peripheral samples (ng/mL), all measurements of LH in hypophyseal portal plasma are reported as concentrations. The concentrations of LH in the hypophyseal portal plasma (ng/mL) were determined by taking into consideration the 0.5 mL of bacitracin in each sample, the recorded estimate of total volume of each sample collected, and the change in hematocrit, measured hourly in the hypophyseal and jugular sample over the course of collection. In addition to these corrections, calculations of GnRH as concentrations (pg/mL) also took into account the factors related to its extraction and reconstitution for assay. These calculations meant that the limit of detection, in terms of concentration, varied for each tube. Thus, the limit of detection expressed as a concentration was calculated for each of the 579 samples and samples with an estimated concentration lower than this were assigned the limit concentration. This occurred with 13.8% of the samples.

Analysis of the Hormonal Time Series

All hormonal series from each ewe were analyzed with the Kushler Brown pulsefit algorithm *(14)*. This nonlinear statistical model assumes exponential decay and attempts to account for the error in the estimates because of biological noise as well as assay error. It identifies pulses by stepwise selection and provides estimates of several parameters of pulsatile secretion including the onset of a pulse, the decay rate, the baseline calculated as the concentration that a hormone would reach in the absence of pulses, and the amplitude of a pulse. Since the algorithm is limited to identifying pulses that are no longer than two observations on the upslope, a postprocessor merges misidentified pulses. The lag time between GnRH and LH pulses was estimated from the pulse starting locations as determined by the Pulsefit algorithm. Further, to assess the temporal relationships between the hormones, the cross correlations at different time lags were calculated between GnRH and portal LH, GnRH and jugular LH, and portal LH and jugular LH. To enhance the precision of estimating the temporal relationships, only those points on or near identified GnRH pulses were used in the analysis. To determine how closely the direct measurements at the hypophyseal portal level can be predicted by deconvolving peripheral measurements, all hormonal series were also analyzed by our implementation (MB) of the Veldhuis et al. deconvolution method *(6,12,19)*. Deconvolution was done with specification of constant variance rather than constant coefficient of variation.

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