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Pulsatile secretion of LH in the ram: a re-evaluation using a discrete deconvolution analysis

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ABSTRACT

The purpose of the present experiment was to characterize LH secretion pulsatility in rams by analysing the instantaneous secretion rate profile obtained by deconvoluting the plasma concentration profile. Plasma LH concentration profiles were obtained by collecting blood samples every 6 min for 24 h during two different sessions separated by an interval of 15 days. Individual kinetic parameters of ovine LH (oLH) were determined following i.v. injection of oLH.

By deconvoluting the plasma concentration profile, it was shown that a pulse has an effective duration of only 20.41 ± 7.69 (S.D.) min whereas the mean duration estimated from measurement of plasma

concentrations was 61.00 ± 15.16 min. The number of pulses was similar before and after deconvolution (7.80 ± 1.99 vs 9.70 ± 3.44 pulses/24 h respectively). Using deconvolution the calculated production rate was 2.26 ± 0.94 $\mu\text{g/kg}$ per 24 h, about 50% of this production being located in the pulses. Statistical analysis of pulsatility revealed that pulse occurrence was a non-periodic event and that the amplitude of LH pulses and the associated amount of LH released were correlated with the duration of the preceding quiescence period, but had no statistically significant influence on the duration of the following quiescence period.

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INTRODUCTION

Progress in the understanding of the mechanisms of luteinizing hormone (LH) secretion and of its gonadal responses should take into consideration the pulsatility paradigm (Atkinson, Bhattacharya, Monroe *et al.* 1970; Bolt, 1971; Katongole, Naftolin & Short, 1971). Thus, accurate characterization of the LH pulse is a prerequisite for any physiological investigation of LH secretion: frequency and rhythm of occurrence, shape and amplitude of pulses are elements that should be subjected to appropriate physiological analysis.

Currently, most studies on LH secretion only take into account profiles of plasma LH concentrations. It should be noted that hormone concentration profiles are not equivalent to hormone secretion profiles, since the concentration is determined by both instantaneous secretion rate and distribution and elimination processes. Consequently, when secretion is the process under investigation, it can be confounded by clearance of the hormone (distribution and elimination), leading

to possible misconceptions when the relationship is investigated either between two hormonal time series or between a hormonal secretion pattern and a given physiological event.

Theoretical solutions to the aforementioned problem have existed for some time (Cutler, 1978) but have only recently been applied to endocrinological investigations. A first approach consists of removing the influence of hormone clearance by deconvoluting the plasma concentration profile using hormone kinetic parameters, obtained in a separate experiment, in which exogenously administered hormone is used and the input signal is controlled (Toutain, Laurentie, Autefage & Alvinerie, 1988). A second approach consists of solving the convolution integral by taking into account secretion and disposition parameters as simultaneous unknowns to be estimated, and thus avoiding administration of exogenous hormone (Veldhuis, Carlson & Johnson, 1987). One constraint in the latter approach is that the form of the input function must be assumed *a priori*; promoters of this method have

selected a random (Gaussian) distribution of instantaneous molecular secretory rate as the best form of the input signal, but other distributions can be considered (Veldhuis *et al.* 1987).

The pulsatility of LH in sheep is well documented (Pelletier, 1983) but has been described only in terms of the LH concentration profile, and no experimental evidence exists concerning the form of the true input signal in rams. The aim of the present experiment was therefore to analyse 24 h concentration profiles of LH by a discrete deconvolution method using rams as a model to describe the true LH pulse signal and to characterize its basic features.

MATERIALS AND METHODS

Animals

Seven 24-month-old rams, of the Préalpes-du-Sud breed, weighing 69.6 ± 5.3 (S.D.) kg were used in June. They were fitted with electrodes to study the relationships between LH secretion and behaviour (results not presented in the present study). After surgery, the rams were placed in individual cages in the same room and were allowed to adapt to the laboratory environmental conditions for at least 3 weeks before the sampling session. Hay was available *ad libitum*, and animals received pelleted food (1 kg/day) daily in two meals at 09.00 and 17.00 h.

Experimental procedure

The day before the sampling session, a heparinized catheter was inserted into each jugular vein. The sampling session began between 08.00 and 09.00 h.

Continuous blood sampling (2 ml every 6 min) was obtained with a withdrawal pump (Minipuls 2, Gilson, Paris, France) connected to one of the jugular catheters. To avoid coagulation, heparinized physiological saline solution was infused (0.2–0.4 ml/min) through the other jugular catheter; a total dose of about 100 000 IU heparin was used during the 24 h sampling period. Blood was kept on a fraction collector for a maximal delay of 2 h, then centrifuged (about 1000 g) and the plasma stored at -20°C until assay.

For each ram, a sampling session was carried out on two occasions separated by 15 days; the first session was in June. About 1 month later, the rams were used for a kinetic LH study. Owing to technical difficulties, the kinetic study was not performed in two of the rams and the results presented will be limited to the five remaining rams. After collection of six control blood samples at 10 min intervals, LH-M3 ovine LH (oLH) equivalent to 1.8 NIH-LH-S1 was injected i.v. at a dose of 6 µg/kg. LH was prepared according to the method described by Jutiz & Courte (1968). The oLH

was dissolved in physiological saline just before administration. Blood samples (5 ml) were collected into heparinized tubes by direct venipuncture (Vacutainer, Becton-Dickinson, Rutherford, NJ, U.S.A.) at 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 60, 75, 90 min and 2, 2.5, 3, 3.5, 4 and 5 h after injection, and were rapidly centrifuged. Plasma was stored at -20°C until radioimmunoassay (RIA) for LH.

LH RIA

A specific, previously described, RIA method was used to measure plasma LH (Pelletier, Garnier, de Reviers *et al.* 1982). All samples were assayed in duplicate in one assay. Results are expressed in terms of LH-M3. The assay sensitivity was 0.31 ng/ml, a concentration approximately two times less than that of the mean basal level. The mean intra-assay S.D. was 4%.

Kinetic analysis

Kinetic analysis of LH plasma concentrations after i.v. administration of oLH was performed using a program for non-linear regression analysis (Yamaoka, Tanigawara, Nakagawa & Uno, 1981); the data points were weighted according to equation 1:

$$\text{Weight} = \frac{1}{\hat{y}_i^2} \quad \text{Equation 1}$$

where $\hat{y}_i$ is the fitted value of the i th observation.

Plasma concentrations for individual rams were fitted to the general polyexponential equation:

$$C(t) = C_0 + \sum_{i=1}^n Y_i \exp(-\lambda_i t) \quad \text{Equation 2}$$

where C represents plasma concentration at time t , C_0 the control concentration, Y_i the coefficient of the i th exponential term, and λ_i the exponent of the i th exponential term. Initial parameter estimates were obtained using the method of the residuals (Gibaldi & Perrier, 1982). The number of exponents (two or three) needed for each data set was determined by application of the Akaike's information criterion (Yamaoka, Nakagawa & Uno, 1978). A biexponential equation was finally selected for all rams. Consequently, data were modelled by a two-compartment open model, with hormone elimination from the central compartment. The parameters (Y_i, λ_i) were used to solve for first-order rate constants of transfer ($k_{1,2}$ and $k_{2,1}$) between central and peripheral compartments according to classic equations (Gibaldi & Perrier, 1982). The volume of the central compartment (V_c) was obtained by dividing the dose by the estimated initial concentration.

The mean residence time (MRT) of LH, which is the mean time required for the LH molecule to transit

through the body and involves a composite of all kinetic processes, was calculated by the linear trapezoidal rule with extrapolation to infinity. The terminal rate constant (λ_2) necessary for an accurate calculation of different moments was obtained from the compartmental analysis. The total plasma clearance (Cl_B) was calculated using equation 3:

$$Cl_B = \frac{\text{Dose}}{AUC_{(0-\infty)}} \quad \text{Equation 3}$$

where $AUC_{(0-\infty)}$ is the total area under the plasma concentration-time curve, calculated by the linear trapezoidal rule with extrapolation to infinity. For both MRT and AUC calculations, control LH concentrations were removed.

The area under the plasma curve for 24 h continuous sampling ($AUC_{(0-24\text{ h})}$) was calculated by the linear trapezoidal rule. The total daily production rate (PR) of LH was calculated by equation 4:

$$PR = AUC_{(0-24\text{ h})} \times Cl_B \quad \text{Equation 4}$$

Instantaneous secretion rate was evaluated using a method of discrete deconvolution, as previously described (Toutain *et al.* 1988). Briefly, a deconvolution analysis is based on modelling the disposition kinetics of the hormone as a linear and time invariant (stationary) system. The LH secretion was modelled by the following convolution integral:

$$C(t) = \int_0^t S(\tau) F(t - \tau) d\tau \quad \text{Equation 5}$$

where $C(t)$ is the concentration of LH at any time t , $S(t)$ is the unknown secretory function (i.e. the input rate of hormone to be identified), $F(t)$ is the response function to a unit impulse, and τ is the dummy variable of integration; $F(t)$ was modelled and parameters identified in a separate experiment (see equation 2 above). Instantaneous secretion rate (raw data and after smoothing) was calculated by solving equation 5. Deconvolution is an ill-conditioned problem due to measurement error in $C(t)$. Consequently, raw data were smoothed with an unweighted least-square spline function (Subroutine EO 2BAF from the NAG library document 135470, NAG Ltd, Oxford, Oxon, U.K.).

Discrete peak detection

Hormonal profiles before deconvolution (plasma concentrations) and after deconvolution (instantaneous secretion rates) were analysed for episodic peak detection using the Pulsar program (Merriam & Wachter, 1982). For plasma concentration profiles, the main criteria selected for this computer analysis were: a constant coefficient of variation describing the relationships between hormone concentrations and intra-assay s.d. (4%), a level of sensitivity of 0.31 ng/ml and peak cut-off

criteria determined empirically. The G values of the Pulsar program were set as follows: $G_1 = 10$; $G_2 = 9$; $G_3 = 8$; $G_4 = 6$; $G_5 = 5$. For the instantaneous secretion rate profile, the G values were $G_1 = 3.8$; $G_2 = 2.6$; $G_3 = 1.8$; $G_4 = 1.5$; $G_5 = 1.2$ and a constant s.d. was selected based on an empirical approach. Indeed, as deconvolution is highly sensitive to data noise, negative values devoid of physiological meaning may be calculated. Taking into account all negative values and the same range of positive values, a normal distribution was observed. It was postulated that these values corresponded to overall noise, and consequently, the s.d. was calculated. In the present experiment, the noise s.d. for the secretion rate values was 0.33–0.54 ng/kg per min depending on individual rams. The following pulse parameters were determined by Pulsar analysis before and after deconvolution: number of peaks per 24 h, mean peak duration, mean interpulse interval (calculated from the onset of a peak to the onset of the following peak), interpulse valley duration (calculated from the end of a peak to the onset of the following peak), amplitude (which was the algebraic difference between maximal peak height and the current basal concentration or basal secretion rate as estimated by Pulsar analysis). Finally, for the entire plasma concentration profile, the area located under the identified pulses was calculated by the linear trapezoidal rule.

Statistical analyses

Kinetic parameters are presented as the mean $\pm$ s.d. except for plasma half-life where the harmonic mean was calculated and its s.d. was computed using a jackknife technique (Lam, Hung & Perrier, 1985). Parameters obtained from Pulsar analysis before and after deconvolution were compared using the Wilcoxon paired sample test.

The distribution function of peak characteristics (duration, amplitude, interpulse intervals) was investigated using the Kolmogorov-Smirnov test which allows comparisons between observed and expected frequencies (Statgraphics, Mercia Software Limited, Birmingham, West Midlands, U.K.). Normal and log-normal distributions were tested.

The relationship between pulse size (amplitude, mass of LH secreted) and the interpulse intervals (either preceding or succeeding) were investigated by linear regression analysis. The results of these correlations were compared with zero by t -testing.

Plasma concentration profile and instantaneous secretion rate profile were subjected to autocorrelation analyses (Systat, Systat Inc., Wilkinson, IL, U.S.A.). Autocorrelation coefficients were estimated between two values separated by a distance of k (lag). In our analyses k (lag) values ranged from 1 to 30 time-units i.e. from 6 to 180 min.

RESULTS

Plasma LH kinetics

Figure 1 shows mean plasma LH concentration ($\mu\text{g/l}$) vs time (min) after the i.v. administration of $6 \mu\text{g}$ oLH/kg to five rams. The initial concentrations observed after injection were high. Thereafter, plasma LH concentrations decreased very rapidly and then returned more slowly to control values within 180 min. The biphasic appearance of the plot was well fitted to a biexponential equation:

$$C_p = Y_1 e^{-\lambda_1 t} + Y_2 e^{-\lambda_2 t} + C_0 \quad \text{Equation 6}$$

where C_p is plasma concentration at time t , Y_1 and Y_2 (ng/ml) are pre-exponential constants, λ_1 and λ_2 (min^{-1}) are exponents and C_0 is the preinjection LH concentration. Consequently, LH disposition was modelled according to a two-compartment model with elimination from the central compartment.

FIGURE 1. Semilogarithmic plot of mean LH plasma concentration vs time after a bolus i.v. administration of ovine LH to five rams. Data are presented as means $\pm$ s.d.

The volume of the central compartment (0.020 ± 0.0039 litres/kg) and the steady-state volume of distribution (0.034 ± 0.015 litres/kg) were very low. The plasma clearance was 0.062 ± 0.013 litres/kg per h. The MRT was 33.5 ± 7.44 min and the mean half-life of the terminal phase was 42.9 ± 11.15 min (harmonic mean). Individual parameter values are presented in Table 1.

LH concentration vs LH secretion rate profiles

The profile of plasma LH concentrations for a representative ram is given in Fig. 2; plasma LH concentrations for the five rams and the two 24 h sampling sessions are presented in Fig. 3a. Visual inspection indicates an episodic pattern of secretion. No significant difference was detected between the two sessions for any of the investigated parameters (paired t -test, $P > 0.05$); pooled values are presented. From the concentration profile, 7.80 ± 1.99 pulses/24 h were identified; this frequency corresponded to a mean interpulse interval of

173.05 ± 107.00 min; the mean duration of a pulse was 61.00 ± 15.16 min giving a total duration of secretory activity of 461.60 ± 116.48 min.

The instantaneous secretion rate levels vs time for the five rams and the two 24 h sampling sessions are depicted in Fig. 3b. Both LH concentration and the corresponding instantaneous LH secretion rate patterns are shown in Fig. 4 for a representative ram. The mean frequency of occurrence of instantaneous LH secretory pulse was higher than the frequency calculated from the plasma concentration profile (9.7 ± 3.4 pulses/24 h) but the difference was not significant (Wilcoxon test, $P > 0.05$). In contrast, the average peak duration (20.41 ± 7.69 min) was only a third of the duration of a peak derived from the concentration profile (61.00 ± 15.16 min, Wilcoxon test, $P < 0.01$), thus indicating that the true total duration of secretory activity was only 186.0 ± 56.6 min, i.e. 2.5 times shorter than the value estimated from the concentration profiles (Wilcoxon test, $P < 0.01$).

Most of the peaks detected from concentration profiles (91%) were also detected from the instantaneous secretion rate profile. In contrast, 28% of the peaks detected from deconvolution profiles were not identifiable from concentration profiles; most of these supplementary secretion peaks corresponded to a splitting of the largest concentration peaks (Fig. 4).

Concentration and instantaneous secretion rate profiles were subjected to autocorrelation analysis. Lag times from 60 to 180 min were tested. The calculated autocorrelation coefficients were not significant. These results suggest that the pulsatility of LH is a non-periodic process, i.e. that LH secretory bursts occur at random.

Distribution of peak characteristics

Polygons of frequency distributions of selected parameters obtained before and after deconvolution are shown in Fig. 5 (peak duration, interpulse interval, interpulse valleys, amplitude, area under the pulse or mass of LH secreted per pulse). None of the frequency distributions was shown to be Gaussian or log-normal (Kolmogorov-Smirnov test, $P < 0.05$). Consequently, mean, median and interquartile range are presented in Table 2.

Taking the five rams into account simultaneously, relationships between the LH pulse amplitude (as the dependent variable) and the preceding valley duration (as the independent variable) were analysed by regression analysis. For concentration and instantaneous secretion rate profiles, highly significant positive correlations, $r = 0.307$ and $r = 0.396$ respectively ($P < 0.01$) were calculated. In contrast, no significant correlation was found between LH pulse amplitude or duration, and the subsequent valley ($r = 0.024$ and $r =$

TABLE 1. Individual values and means ($\pm$ S.D.) of the kinetic parameters describing ovine LH (oLH) disposition in plasma after bolus administration of oLH (6 μ g/kg) to five rams

Parameters	Ram 1	Ram 2	Ram 3	Ram 4	Ram 5	Mean $\pm$ S.D.
Y_0 (ng/ml)	324.70	225.41	336.19	304.65	328.63	303.92 $\pm$ 45.41
Y_1 (ng/ml)	295.10	199.58	299.46	264.21	263.39	264.35 $\pm$ 39.34
Y_2 (ng/ml)	28.27	19.65	34.10	37.13	59.56	35.74 $\pm$ 14.89
C_0 (ng/ml)	1.33	6.18	2.63	3.31	5.68	3.83 $\pm$ 2.06
λ_1 (min $^{-1}$)	0.0619	0.0686	0.071	0.076	0.100	0.0755 $\pm$ 0.015
λ_2 (min $^{-1}$)	0.01218	0.0212	0.0119	0.0162	0.0192	0.01614 $\pm$ 0.00414
$t_{1/2}$ (min)	56.88	32.66	58.23	42.77	36.03	42.91 $\pm$ 11.15*
MRT (min)	37.74	22.43	42.40	32.46	32.69	33.54 $\pm$ 7.44
Cl_b (l/h per kg)	0.050	0.083	0.052	0.061	0.064	0.062 $\pm$ 0.013
V_{ss} (l/kg)	0.032	0.035	0.036	0.034	0.034	0.034 $\pm$ 0.015
V_c (l/kg)	0.018	0.027	0.017	0.020	0.019	0.020 $\pm$ 0.0039
$k_{1,0}$ (min $^{-1}$)	0.0456	0.05716	0.0471	0.0523	0.0563	0.0517 $\pm$ 0.0052

Y_0 , initial concentration; Y_1 and Y_2 , pre-exponential constants; λ_1 and λ_2 , exponential constants for the biexponential equation describing the plasma concentration time curve; C_0 , basal control concentration; $t_{1/2}$, plasma half-time; MRT, mean residence time; Cl_b , body clearance; V_{ss} , steady-state volume of distribution; V_c , volume of central compartment; $k_{1,0}$, first-order rate constant of elimination from the central compartment.

*Harmonic mean.

FIGURE 2. Representative 24 h pattern of LH plasma concentration in a representative ram. Plasma LH concentrations were measured by radioimmunoassay of samples obtained every 6 min for 24 h.

0.041, $P > 0.05$ for concentration and instantaneous secretion rate profiles respectively).

The mean concentration amplitude was 4.55 ± 2.54 ng/ml which represents an increment of about 600% over the estimated current basal concentration. The mean production rate amplitude was 11.85 ± 9.3 ng/kg per min. The calculated mass of LH released per secretory pulse was 146 ng/kg from the concentration profile and 125 ng/kg from the instantaneous secretion rate profile; the difference was not significant (paired t -test, $P > 0.05$).

For both concentration and secretion rate profiles, linear regression analysis between mass of LH secreted during a pulse (independent variable) and duration of the preceding valley (dependent variable) revealed a significant positive correlation ($r = 0.296$, $P < 0.05$ for concentration profile, and $r = 0.383$, $P < 0.05$ for instantaneous secretion rate profile). In contrast, valley duration was not significantly correlated with the mass of LH released in the preceding pulse ($r = 0.032$ for concentration profile, and $r = 0.053$, $P > 0.05$ for instantaneous secretion profile). These results suggest that

FIGURE 3. 24 h pattern of LH secretion profile for five rams obtained in two different sampling sessions. (a) 24 h plasma concentration profile. Plasma LH concentrations were measured by radioimmunoassay of samples obtained every 6 min for 24 h. (b) 24 h instantaneous secretion rate (ISR) profile. The instantaneous rates of LH secretion were reconstructed by the deconvolution method (negative values are not presented).

secretory events (amplitude, mass of released material) do not modify the duration of the secretory quiescence following a given pulse, but that the duration of this pause influences the size of the subsequent pulse.

Production rate of LH

The total production rate estimated by deconvolution was $2.26 \pm 0.94 \mu\text{g/kg per 24 h}$. This value was not significantly different from that obtained using plasma clearance and the 24 h overall mean LH concentration

($2.09 \pm 0.74 \mu\text{g/kg per 24 h}$; Wilcoxon test, $P > 0.05$; Table 3).

Visual inspection of 24 h profiles of instantaneous secretion rate (Fig. 3) suggests that pulsatile LH secretion may be superimposed on baseline LH secretion. From deconvolution, the total LH secreted during pulses was $1.24 \pm 0.74 \mu\text{g/kg per 24 h}$. Consequently, the secretion not located in the identified pulses totalled $1.02 \pm 0.27 \mu\text{g/kg per 24 h}$, i.e. 47.80% of the total 24 h production rate. When the plasma concentration profile was considered, the total area

FIGURE 4. Representative 24 h pattern of LH plasma concentration (thin line) and corresponding instantaneous LH secretion rate (ISR, thick line) in a representative ram. Plasma LH was measured by radioimmunoassay of samples obtained every 6 min for 24 h. Instantaneous secretion rates were reconstructed by a deconvolution method.

under the peaks was 55.65% of the 24 h area. Consequently, secretion not located in pulses was estimated to be 44.35% of the total production. These values, whether obtained from the concentration or instantaneous secretion rate profiles, were not statistically different (paired *t*-test, $P > 0.05$).

DISCUSSION

In the present study, the 24 h instantaneous secretion rate profile of LH in rams has been reconstructed by discrete deconvolution from the 24 h plasma concentration profile obtained in June, a month when LH pulsatility has been shown to be high in the ram (Pelletier, Garnier, de Reviers *et al.* 1982). The main results are as follows: (i) the actual duration of the LH secretory pulse is much shorter (about 20 min) than that derived from plasma LH concentration profiles (about 60 min); (ii) only about half of the calculated LH secretion takes place during pulses (phasic secretion); (iii) LH pulsatility is apparently a non-periodic process; and (iv) the size of a given secretory pulse depends on the duration of the preceding pulsatile secretion quiescence, but does not influence the duration of the subsequent quiescent period.

An appropriate sampling frequency, in agreement with the disposition parameters of LH, must be selected to characterize LH secretion pulsatility. In man, it has been reported that a sampling interval of 5–7 min is best for the detection of LH peaks

(Veldhuis, Evans, Johnson *et al.* 1986a). Because of the similarity of LH clearance in man (Veldhuis, Fraioli, Rogol & Dufau, 1986b) and ram, a 6-min sampling interval was used in the present experiments. It must be noted that as blood was continuously drawn from an automatic pump, then this involves a form of biological smoothing before sampling and it is likely that this procedure results in an underestimate of the true maximal concentration of an LH peak.

As pointed out in the introduction, the 24-h instantaneous secretion rate profile, as reconstructed in the present study by a discrete deconvolution method from the 24-h plasma concentration profile, needs no *a priori* assumption of the shape of the secretory pulse, while hormone clearance parameters were determined in a separate experiment using exogenous hormone. In the present experiment, we made no assumptions about the structure of the input function, because the primary objective of our deconvolution analysis was to describe precisely the input function. Another approach would have been to model the secretory process during a pulse according to a Gaussian distribution of instantaneous molecular secretory rate, and to solve the convolution integral by considering secretion and clearance parameters as unknown (Veldhuis *et al.* 1987). Our results support the validity of the approach used in the present study, since there was evidence of an apparent tonic secretion (see below). One limitation of the present analysis is the calculation of some negative input rates which are devoid of physiological meaning. Indeed, deconvolution is an ill-conditioned problem

FIGURE 5. Frequency polygons of some peak characteristics estimated from concentration profiles (a) or instantaneous secretion rate (ISR) profiles (b). Plasma LH concentrations were measured by radioimmunoassay of samples obtained from five rams in two separate 24 h sampling sessions every 6 min. Instantaneous secretion rates were reconstructed by a deconvolution method.

that is very sensitive to data noise. Consequently, negative values may be calculated when the secretion rate is close to zero. To minimize this drawback, raw data were smoothed by a spline procedure. Nevertheless negative values were still obtained but their frequency was minimal (7.5% of all data points) and the estimated noise (0.42 ± 0.078 ng/kg per min) was only 3.5% of the peak mean amplitude.

Validity and usefulness of disposition parameters from discrete deconvolution analysis depend on the similarity between the administered material and the hormone actually released. In addition, deconvolution analysis is based on modelling the disposition kinetics of the hormones as a linear system. In the rat, the disposition of [^3H]oLH has been shown to be linear (Ascoli, Liddle & Puett, 1975). In contrast, non-linearity has been demonstrated for immunoreactive LH (ir-LH) in man (Veldhuis, Fraioli, Rogol & Dufau, 1986b). In the present experiment, we assumed oLH linearity, on the basis of experimental evidence reported in the literature. A mean plasma half-life of 32 min was calculated using a tracer dose of ^{131}I -labelled LH in rams (De Krester, Atkins & Paulsen,

1973). After administration of $0.52 \mu\text{g}$ ^{131}I -labelled LH, a half-life of 43 min was reported (Akbar, Nett & Niswender, 1974), a value identical with that found in the present experiment (43 min) after injection of a single dose of $6 \mu\text{g}$ oLH/kg, corresponding to about three times the daily production rate. In addition, the half-life of disappearance of LH, approximated from the last three data points of endogenous LH peaks, indicates a terminal half-life of about 46 min (data not shown). Estimated plasma clearance was 0.062 litres/kg per h; the value is low but similar to that reported for ir-LH in man (about 0.044 litres/kg per h) (Veldhuis *et al.* 1986b).

When instantaneous secretion rate is considered, pulses seem to occur with an average interval of 140 min, exhibit a mean duration of 20 min and are associated with the release of 125 ng oLH/kg. The duration of the pulse is only a third of that calculated using the corresponding plasma concentration profile. Similar results have been obtained in man where 95% of ir-LH have been shown to be released within 15 min, during a spontaneous secretory episode, while the apparent duration of the peak estimated from the

TABLE 2. Peak characterization from plasma LH concentration (C) and instantaneous LH secretion rate (ISR). Data presented are means $\pm$ S.D., median and interquartile range and were obtained from five rams in two separate sessions

	Mean $\pm$ S.D.	Median	Interquartile range
Number of peaks (per 24 h)			
C	7.8 $\pm$ 1.99	8	6
ISR	9.7 $\pm$ 3.43	9	10
Amplitude			
C (μ g/l)	4.55 $\pm$ 2.54	3.87	4.47
ISR (ng/kg per min)	11.85 $\pm$ 9.29	7.25	11.71
Mass of LH secreted in pulses (ng/kg)			
C	146.21 $\pm$ 122.18	137	189.78
ISR	125.34 $\pm$ 152.15	72	155.1
Peak duration (min)			
C	61.00 $\pm$ 15.16	60.88	42
ISR	20.41 $\pm$ 7.69	16.55	24
Total secretory activity (min per 24 h)			
C	461.60 $\pm$ 116.48	424	342
ISR	186.00 $\pm$ 56.56	195	186
Interpulse interval (min)			
C	173.05 $\pm$ 107.00	177	180
ISR	140.41 $\pm$ 108.01	126	198
Valley duration (min)			
C	99.91 $\pm$ 98.72	84	174
ISR	113.59 $\pm$ 106.78	96	192

Parameters were obtained directly by Pulsar analysis (number of peaks, amplitudes, peak duration and total secretory activity). The interpulse interval is the duration between the onset of a peak to the onset of following peak. Valley duration is the time elapsed between the end of a peak to the onset of the next peak.

TABLE 3. Mean $\pm$ S.D. daily ovine LH (oLH) plasma concentration and calculated LH production rate from the concentration profile (C) and instantaneous secretion rate (ISR) in the same five rams in first, second and for both periods of the experiment

Parameters (units)	1st period	2nd period	Both periods
AUC _(0-24 h) (μ g/l per min)	1864.7 $\pm$ 460.4	2131.3 $\pm$ 569.5	1998.0 $\pm$ 508.1
Mean daily LH conc. (μ g/l)	1.29 $\pm$ 0.32	1.48 $\pm$ 0.40	1.39 $\pm$ 0.35
Production rate from C (μ g/kg for 24 h)	1.92 $\pm$ 0.55	2.27 $\pm$ 0.99	2.09 $\pm$ 0.74
Production rate from ISR (μ g/kg for 24 h)	2.03 $\pm$ 0.65	2.49 $\pm$ 1.28	2.26 $\pm$ 0.94

AUC is the area under the LH concentration vs time curve.

concentration profile was about 60 min (Veldhuis & Johnson, 1988), i.e. a value close to that obtained here for the ram. Physiological consequences of these differences are relevant when factors controlling the duration of a secretory episode are discussed. Indeed, it has been demonstrated that major LH pulses are systematically coupled with a pulse of LH-releasing hormone (LHRH) release by the hypothalamus. In ovariectomized ewes chronically implanted with cannulae for portal blood collection, it was shown that

the LHRH pulse was much shorter (Caraty, Locatelli & Martin, 1989) than the LH pulse as determined from a plasma concentration profile. This is consistent with the results of the present experiment and it can be suggested that the terminal phase of an LH pulse is directly related to the drop in portal LHRH concentration. Another point of interest concerns the supplementary secretion peaks corresponding to splitting of the largest concentration peaks (see Fig. 4). The deconvolution technique renders these peaks still more evident, but their origin

and meaning remain unclear as, in contrast with the major LH pulse, they do not appear to be associated with LHRH release (see Fig. 1 in Caraty *et al.* 1989).

It is generally assumed that testosterone is involved in the control of the pattern of LH secretion in the male. In the ram, secretory peaks of LH induce secretory peaks of testosterone and the interval between the peak concentration of LH and that of testosterone is about 45 min (Terqui, Garnier, de Reviers *et al.* 1980). Such a lag time indicates that a negative feedback effect of testosterone cannot explain the relatively rapid termination of the LH secretion pulse.

In the ram, the occurrence of the LH pulse is apparently non-periodic, a finding which fits with data showing that pulse interval sequence is a renewal process in man (Butler, Spratt, O'Dea & Crowley, 1986). Furthermore regression analyses show the absence of a relationship between pulse amplitude (or secreted LH mass) and duration of the succeeding secretory quiescence. In contrast, a significant positive influence of quiescence duration on the amplitude of the subsequent pulse can be suggested. It can be hypothesized that a long secretory quiescence may increase the LH mass released in the subsequent pulse, e.g. by increasing the size of the readily releasable LH pool.

In the present experiment, an apparent tonic secretion was calculated by deconvoluting plasma concentration close to our analytic level of quantification, and the reality of a tonic secretion merits discussion. In man, LH secretion between pulses appears to be limited to about 10% of the total secretion (Urban, Evans, Rogol *et al.* 1988). Using a deconvolution method and fixing the input signal to a Gaussian process, it was concluded that a tonic mode of LH secretion interposed between pulse secretory episodes was not necessary to describe the LH concentration profile accurately (Veldhuis, Johnson & Dufau, 1989). In the present experiment, no statistical effort (e.g. removal of basal noise from the calculated tonic secretion) was made to establish the reality of tonic secretion. It was considered that evidence should be obtained solely from biological studies (e.g. by improving analytical methods, use of in-vitro cell culture, etc.) and not from statistical analysis (the conclusion of which is largely influenced by the formulation of a null hypothesis, i.e. existence or not of a basal secretion) nor from the fact that good fitting can be obtained with models which exclude, *a priori*, any basal secretion. The existence of tonic secretion in the present experiment merits further investigation, as about 50% of the calculated LH secretion was not located within identified pulses. Possible basal LH secretion is consistent with in-vitro results which demonstrate that LH release from the isolated human pituitary is characterized by high-frequency low-amplitude pulsatile secretion, independent of hypothalamic stimulation (Gambacciani, Liu,

Swartz *et al.* 1987). Such continuous LH release would explain why a long interval between two LH secretory episodes does not reduce peripheral plasma concentrations to undetectable levels.

In conclusion, it is clear from the result of the present experiment that to consider plasma secretion rather than secretion rate may be misleading when LH secretion is the process under investigation. The main potential drawback is the duration of an episode of LH secretion which is much shorter when secretion rates are considered. In addition, concentration profiles give no information on the mass of LH which is actually secreted (phasic vs tonic secretion). Of course, concentration profiles remain the independent variable of interest when a physiological effect of LH is documented, since effects are dependent on plasma concentrations and not on secretion rate.

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