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Instantaneous Secretion Rate of Growth Hormone in Lambs: Relationships with Sleep, Food Intake, and Posture*

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ABSTRACT. Relationships among sleep, feeding behavior, posture, and GH secretion were investigated in two groups of ruminant lambs in January ($n = 6$) and May ($n = 3$). Lambs were placed in individual cages and fed *ad libitum*. Behavioral features were obtained from continuous polygraphic recording. Blood was collected from undisturbed sheep every 3 min for 24 h via an indwelling catheter connected to a peristaltic pump. One month after the sampling session, ovine GH (oGH) was iv administered to evaluate oGH kinetic parameters. From GH plasma concentrations and oGH kinetic parameters, the instantaneous secretion rate of GH was reconstituted using a numerical deconvolution method.

All lambs exhibited normal behavioral patterns. The clearance of oGH was similar for the two groups, and the daily production rates of GH were estimated at $14.60 \pm 7.99 \mu\text{g/kg} \cdot 24 \text{ h}$ in January and $10.57 \pm 5.21 \mu\text{g/kg} \cdot 24 \text{ h}$ in May. Analysis of concentration profiles indicate an episodic pattern of GH secretion into plasma. The mean number of peaks was $16.22 \pm$

$4.47/24 \text{ h}$, and the mean duration was $47.2 \pm 12.8 \text{ min}$ for the nine sheep. When instantaneous secretion rates were taken into account, the number of identified peaks was similar, but the mean duration was reduced ($32.9 \pm 9.8 \text{ min}$ for the nine sheep).

Significant relationships were not found between GH plasma concentration profiles and the state of vigilance, food behavior, or posture. Conversely, when the instantaneous secretion rates were taken into account, the highest GH production rate was detected during rest, i.e. slow wave sleep and rapid eye movement sleep, absence of food intake or rumination, and lying down. It is emphasized that the use of GH instantaneous secretion rate instead of GH concentration is of importance when evaluating the relationships between GH dynamics and short duration events. It is concluded that the influence of vigilance on GH secretion, which has already been demonstrated in humans, is likely to exist in other species (*Endocrinology* 125: 642-651, 1989)

THE EPISODIC secretion of GH is enhanced during sleep in man, especially during the first episode of slow wave sleep (SWS) (1, 2). In contrast, no relationship has been evidenced between GH secretion and sleep-wakefulness cycles or a particular sleep stage in rhesus monkey and dog (3, 4). In the adult rat, initial studies have been unable to demonstrate a correlation between GH and sleep phase (5). However, more recent studies indicate that GH secretion in the immature rat is sleep related, with a significant correlation between GH concentration and the duration of sleep during the preceding 10 min (6). In the adult male rat, the GH secretory peaks have been shown to occur with a 3-h periodicity and to be related to the early stage of each sleep cycle, with a lag time of 70 min (7). In goats, GH release is not associated with a particular sleep stage, as assessed by

electroencephalographic recording (8). In pregnant and ovariectomized ewes (9, 10), a nocturnal increase in GH has been reported, but other studies in both male sheep (11) and anestrus ewes (12) failed to demonstrate a GH rhythm related to the time of day. In dairy cows and heifers, no difference between diurnal and nocturnal GH secretion has been detected, while in calves GH concentrations were higher during the day than at night (13). Similarly, darkness produces a decrease in GH concentration in the growing pig (14). Finally, a temporal relationship between GH secretion and a particular stage of sleep (SWS) has been demonstrated only in man. However, if GH release is related to the mechanism of SWS or if GH and the sleep-wakefulness cycle have their own but synchronous pacemakers remain open to questions.

Relevant information from animals for which sleep is typically polyphasic (broken by more or less long episodes of wakefulness) should help to evaluate the possible relationships existing between GH secretion, sleep-wakefulness cycle, and circadian rhythms.

When studying relationships between GH secretion

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and sleep-wakefulness cycle in animals, it must be noted that the sleep pattern is much more fragmented in animals (15) than in man. The sleep cycle, *i.e.* the interval between the end of two rapid eye movement (REM) sleep episodes separated by an episode of SWS, is about 100 min in man, but only 10–20 min in animal species, including rodents, carnivores, and ruminants (16). Consequently, to demonstrate any relationship between GH secretion and a particular state of vigilance, a first requirement to characterize very short term GH plasma concentration pulsations is an intensive venous sampling schedule. The influence of frequent sampling on the detected frequency of pulsation of different hypophyseal hormones has been documented (17); a sampling interval significantly shorter than the intended duration of an episode of sleep is a first requirement for such a study.

A second point deserves attention. The plasma level of any hormone is not only dependent on its secretion rate, which is the process under investigation, but also on its distribution and elimination. Therefore, due to the relatively long plasma elimination half-time of GH (which is 40 min in sheep) (18) with respect to a sleep episode (10 min), a high GH concentration may be measured during a given stage of sleep despite the absence of any secretion. This is a major drawback which can impair any correlation studies between GH secretion dynamics and sleep stage in all animal species for which the sleep pattern is much more fragmented than in man. The solution to the aforementioned problem exists. It consists of using deconvolution, namely a mathematical analysis that removes the influence of distribution and elimination processes in order to reconstruct the actual GH instantaneous secretion rate from the plasma concentration profile (19, 20).

The aim of the present experiments was to investigate the influence of the state of vigilance, feeding behavior, and posture on the GH secretion rate in lambs, using both an intensive venous sampling schedule and deconvolution analysis of GH concentration profiles.

Materials and Methods

Animals

Ruminant lambs (three males and six females) of the Merinos d'Arles breed were 4–5 months old and weighed 31.8 ± 2.8 kg on the day of the experiment. About 1 month before the sampling session, electrodes for recording the electrocorticogram (EcoG), electrooculogram (EOG), and masticatory movements were inserted under thiopentone anesthesia and aseptic conditions. The electrodes were silver-coated screws attached to stainless-steel wire. Four EcoG electrodes were screwed into the skull approximately 10 mm from each side of the sagittal suture and over the parietal and occipital regions of the cerebral cortex. The EOG electrodes were placed into the temporal bone and the lacrymal bone. An electrode was inserted over the nasal

bone in order to record masticatory movements. Electrodes were fixed and insulated with an acrylic resin.

After surgery, the lambs were placed in individual cages located in the same room and were allowed to adapt to the laboratory environmental conditions until the sampling session took place. They were fed hay *ad libitum*, given daily in two meals at 0900 and 1700 h, and received pelleted food (400 g) in a daily single meal at 0830 h. This ration was considered to provide enough energy and nitrogen for maintenance and moderate growth; water was provided *ad libitum*.

Group allocation and experimental procedures

The lambs were studied in two groups. The first group ($n = 6$, two males and four females) was investigated in January, and the second group ($n = 3$; one male and two females) in May. For both seasons, the natural photoperiod was respected.

Twenty-four hours before the sampling session, two catheters containing heparin were inserted into the jugular veins. In addition, electrodes for recording electromyograms made of stainless-steel wire (0.5 mm in diameter) were sutured into the superficial dorsal muscle of the neck (Splenius, brachiocephalicus). The position of the animal (standing *vs.* sternal recumbency) was registered using photoelectric cells (infrared beam). The electrophysiological data were recorded on a multichannel electroencephalograph (Alvar, Paris, France) at a paper speed of 100 mm/min. Time constants of 0.3 s for EcoG, 0.1 for electromyograms, and 1 s for EOG and masticatory movements were selected. Filters were used for EOG and masticatory movements to eliminate high frequency signals.

Continuous blood sampling (0.8 ml every 3 min) was obtained with a withdrawal pump (Minipuls 2, Gilson, Paris, France) connected to a jugular catheter. To avoid coagulation heparinized physiological saline 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. The blood was kept on a fraction collector for a maximal time of 2 h, then centrifuged, and the plasma was stored at -20°C until assay.

About 1 month later, *i.e.* in February for group 1 and June for group 2, six control blood samples were obtained at 10-min intervals, and ovine GH (oGH) (NIH GH.S5; 1 IU/mg) was administered at a rate of $18.5 \mu\text{g/kg}$ for the first group and $50 \mu\text{g/kg}$ for the second group. Just before administration, oGH was dissolved in physiological saline solution containing 1% BSA. Blood samples (5 ml) were collected into heparinized tubes at 1, 2, 4, 8, 15, 30, 60, and 90 min and 2, 3, 4, and 5 h postinjection, and were rapidly centrifuged. Plasma was stored at -20°C until analysis.

GH RIA

oGH was measured by specific RIA, using a double antibody separation method. Reagents for the oGH assay were supplied by the National Hormone and Pituitary Program (NIDDK, Bethesda, MD), except the second antibody (sheep antirabbit γ -globulin) which was prepared in our laboratory. Assay sensitivity was 0.5 ng/ml, and the intraassay coefficient of variation was 5%. All samples from a given experiment were run in the same assay at the appropriate dilution.

Polygraphic scoring procedure

Records were divided into 1-min consecutive sections and were scored visually. According to the EcoG pattern, eye movements, and muscle tone, the following states of vigilance were distinguished: I) wakefulness: desynchronized low voltage fast activity EcoG, positive tone in neck muscle, and eye movements; II) drowsiness: a combination of desynchronized low voltage and synchronized high voltage activities, muscle tone presence, and infrequent eye movements; III) SWS, characterized by synchronized high voltage slow activity in EcoG with low tone in the neck muscle; and IV) REM sleep with desynchronized low voltage fast activity EcoG, rapid eye movement evaluated from EOG, and a total absence of muscle tone (21). The study of jaw movements permitted the identification of three types of feeding behavior: rest, food intake, and rumination. Position was classified as standing up or lying down according to the photoelectric signal.

Kinetic analysis

Pharmacokinetic analysis of oGH concentration after iv administration was performed using a program for nonlinear regression analysis (22); the data points were weighted according to the Eq I:

$$w_i = 1/y_i^2 \quad (I)$$

where w_i is the weight, and y_i is the fitted value of the i th observation.

Plasma concentrations of individual sheep were fitted to the general polyexponential Eq II:

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

In Eq II, C represents plasma concentration at time t , C_0 the basal concentration, Y_i is the coefficient of the i th exponential term, and λ_i is the exponent of the i th exponential term. Initial parameter estimates were obtained using linear regression methods. The number of exponents (two or three) needed for each data set was determined by application of the Akaike's information criterion (23). A biexponential equation was selected for all sheep. Consequently, data were described by a two-compartmental 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_{12} and K_{21}) between central and peripheral compartments according to classical equations (24). 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 oGH, which is the mean time for the oGH 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 the equation:

$$Cl_B(iv) = \text{dose}/AUC_{(0-\infty)} \quad (III)$$

In Eq III, $AUC_{(0-\infty)}$ is the total area under the plasma concen-

tration-time curve, calculated by the linear trapezoidal rule with extrapolation to infinity. For both MRT and AUC calculations, basal GH concentration was taken into account.

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 GH was calculated by Equation IV:

$$PR = AUC_{(0-24 \text{ h})} \cdot Cl_B \quad (IV)$$

In Eq IV, Cl_B is the body oGH clearance and $AUC_{(0-24 \text{ h})}$ the total area under the 24-h curve.

Instantaneous secretion rate was evaluated using a method of discrete deconvolution, as previously described (19). Briefly, a deconvolution analysis is based on modelling the disposition kinetic of the hormone as a linear and time invariant (stationary system). The GH secretion was modelled by the following convolution integral (Eq V)

$$C(t) = \int_0^t F(t - \tau) \cdot S(\tau) \cdot d\tau \quad (V)$$

where $C(t)$ is the measured plasma concentration of GH at any instant in time t , $S(\tau)$ is the unknown function (i.e. the input rate of hormone which is to be calculated), $F(t)$ is the response function to a unit impulse, and τ is the integration variable; $F(t)$ was identified in a separate experiment (see Eq II above) solving Eq V for $S(\tau)$ given $C(t)$ and $F(t)$. Deconvolution is an ill conditioned problem due to measurement error in $C(t)$. To improve the situation, raw data were smoothed with an unweighted least square spline function (Subroutine EO 2BAF from the NaG library, document 135470).

Hormonal profiles before deconvolution (plasma concentrations) and after deconvolution (instantaneous secretion rates), were analyzed for episodic peak detection using the PULSAR program (25). For the plasma concentration profile the main criteria selected for this computer analysis were: a constant coefficient variation (5%) to describe the relationship between hormone concentration and intraassay SD, a level of sensitivity of 0.5 ng/ml, and peak cut-off criteria determined empirically. For the instantaneous secretion rate profile, a constant SD was selected based on an empirical approach. Indeed, as deconvolution is highly sensitive to data noise, negative values devoid of any physiological meaning can be calculated. Taking into account all negative values and the corresponding symmetric positive values, a normal distribution was observed. It was postulated that these values corresponded to noise, and consequently, the SD was calculated. In the present experiment, the SD for the secretion rate values was between 0.9–4.5 ng/kg·min depending on individual sheep; the overall mean coefficient of variation of noise was estimated to be $27.2 \pm 12.1\%$.

Statistical analyses

Data are presented as the mean $\pm$ SD. For plasma half-life, harmonic means were calculated, and their SDs were computed using a jackknife technique (26). Comparison of parameters obtained in the two groups of sheep was performed using Mann-Whitney U test. Comparison of results obtained from PULSAR analysis before and after deconvolution was carried out using a Wilcoxon paired sample test. A nonparametric analysis of

variance (Friedman test) was used to estimate the effects of the different behaviors on the plasma GH concentrations and on their corresponding instantaneous secretion rate.

Results

Sleep patterns and related phenomena

All sheep exhibited a regular cyclic pattern of rest and activity. Figure 1 shows the hypnogram, posture, and feeding behavior and the corresponding variation in GH concentrations for a representative lamb. Typically, and mostly during the dark period, each sleep cycle began as the animal lay down; then, the lamb became drowsy and entered SWS. A single period of SWS lasted approximately 10 min. It was followed by a period of REM sleep lasting about 4–6 min, which ended in arousal. REM sleep was not univocally identified in two of the nine lambs, and this state was ignored in the correlation analysis for these two sheep. The average percentages for the 24 h were $70.09 \pm 10.50\%$ for wakefulness, $11.53 \pm 7.55\%$ for drowsiness, $17.32 \pm 4.79\%$ for SWS, and $1.04 \pm 0.88\%$ for REM sleep.

As food was given *ad libitum*, food intake ($18.43 \pm 5.48\%$ of the 24 h) occurred throughout the nycthemer, but with the two most important intakes connected with meals in the morning and evening. Occasionally, lambs were observed to ruminate while standing awake, but most often during drowsiness or SWS. Rumination was

predominantly observed during the night, and its duration was $24.14 \pm 7.87\%$ of the 24 h; no feeding activity was observed during $57.42 \pm 10.05\%$ of the nycthemer. In a confinement cage, laying down was much more frequent than standing (72.09 $\pm$ 10.94% *vs.* 27.91 $\pm$ 10.94%). Values for the different behavioral parameters are presented in Table 1.

oGH kinetic

Mean plasma oGH concentrations after iv injection of oGH for the two groups of lambs are shown in Fig. 2. After oGH administration, the initial observed concentrations were higher (418.9 ng/ml) for the group given 18.5 μ g/kg than for the group given 50 μ g/kg (325.3 ng/ml). Then, GH plasma concentrations decreased very rapidly and returned more slowly to control values within 3–5 h. The biphasic appearance of the plot was well fitted by a biexponential equation corresponding to a two-compartment open model with elimination from central compartment. Kinetic parameters that describe the disposition of oGH calculated from data obtained in each lamb are given in Tables 2A and 2B.

The mean body clearances were similar ($P > 0.05$) for the two groups (0.211 ± 0.106 and 0.163 ± 0.032 liter/h·kg for sheep sampled in May and January, respectively). In contrast, the volume of the central compartment appeared to be significantly lower ($P < 0.05$) in January

FIG. 1. Representative 24-h pattern of GH plasma concentration, vigilance, feeding behavior, and posture in a lamb. Plasma GH was measured by RIA on samples obtained every 3 min for 24 h; behavioral features were assessed by polygraphic recording. Sleep stage: 1, Wakefulness; 2, drowsiness; 3, SWS; 4, REM. Feeding behavior: 1, No food intake; 2, food intake; 3, rumination. Posture: 1, Standing up; 2, lying down.

TABLE 1. Duration of behavioral events (vigilance parameters, feeding, and posture) in the course of the 24 h of the experiment

Behavior	Duration (min)
Wakefulness	981.70 $\pm$ 169.70
Drowsiness	160.44 $\pm$ 104.13
SWS	240.77 $\pm$ 63.47
REM	14.27 $\pm$ 12.03
No food intake	801.67 $\pm$ 142.36
Food intake	256.33 $\pm$ 70.36
Rumination	339.22 $\pm$ 117.41
Standing	388.22 $\pm$ 145.95
Lying down	1009.00 $\pm$ 165.46
Total	1397.22 $\pm$ 60.23
Missing	42.78 $\pm$ 60.23

Values are the mean $\pm$ SD for the nine lambs under study.

FIG. 2. Semilogarithmic plot of GH plasma concentration vs. time curves after a bolus iv administration of oGH to lambs. A, Mean $\pm$ SD for the six lambs of the January group given oGH at a dose of 18.5 µg/kg. B, Mean $\pm$ SD values for the three lambs of the May group given 50 µg/kg oGH.

(0.046 $\pm$ 0.006 liter/kg) than in May (0.158 $\pm$ 0.028 liter/kg). The MRT was significantly different ($P < 0.05$) for sheep sampled in May (80.34 $\pm$ 26.88 min) and January (36.40 $\pm$ 7.09 min).

The calculated daily production rate was 10.57 $\pm$ 5.21 µg/kg·24 h for the group sampled in May and 14.60 $\pm$ 7.99 µg/kg·24 h for the group in January; the difference was not significant. Individual values are given in Tables 3A and 3B.

GH concentration vs. GH secretion rate profiles and pulse characterization

Visual inspection of concentration profiles (Fig. 1) suggested an episodic pattern of GH secretion into plasma. Analysis of plasma concentration data using the PULSAR program showed no significant difference between the two groups for the number of peaks (16.22 $\pm$ 4.47 peaks/24 h; $n = 9$), the peak duration (47.24 $\pm$ 12.82 min; $n = 9$), and the resulting total duration of secretory activity (726.03 $\pm$ 104.4 min). In contrast, the mean amplitude of peaks was significantly lower ($P < 0.02$) for sheep sampled in May (2.5 $\pm$ 0.2 ng/ml; $n = 3$) than for those sampled in January (6.6 $\pm$ 3.6 ng/ml; $n = 6$). Data for both groups are given in Table 4.

Analysis of instantaneous secretion rates by PULSAR showed a significant difference ($P < 0.02$) only for the peak duration, which was shorter in May (25.02 $\pm$ 2.93 min; $n = 3$) than in January (36.83 $\pm$ 9.68 min; $n = 6$; Fig. 3). Other parameters were similar for the two groups ($P > 0.05$), i.e. the number of peaks (15.67 $\pm$ 4.61; $n = 9$), the peak amplitude (3.05 $\pm$ 1.38 ng/kg·min; $n = 9$), and the total duration of secretory activity (525.96 $\pm$ 264.1 min; $n = 9$).

Comparison of plasma concentration data for the nine lambs before and after deconvolution showed significant reduction ($P < 0.01$) of the peak duration after deconvolution (32.90 $\pm$ 9.78 vs. 47.24 $\pm$ 12.82 min). In contrast, the number of peaks was not significantly different before (16.22 $\pm$ 4.47) and after deconvolution (15.67 $\pm$ 4.61). Data for both groups are given in Table 4.

The total production rate estimated by deconvolution was not significantly different for the group sampled in May (12.10 $\pm$ 3.92 µg/kg·24 h) and the group in January (14.72 $\pm$ 7.58 µg/kg·24 h). In addition, these values were not significantly different from those obtained using body clearance and the overall mean GH 24-h concentration (Tables 3A and 3B).

GH concentration vs. GH secretion rate profiles and behavior

The mean plasma concentration of GH and the mean instantaneous secretion rates for the nine lambs and the nine behavioral states investigated are given in Table 5.

TABLE 2A. Individual values and mean ($\pm$ SD) of the kinetic parameters describing oGH disposition in plasma after iv bolus administration of oGH (18.5 μ g/kg) to the six lambs studied in January

Parameters	A	B	C	D	E	F	Mean $\pm$ SD
Y ₀ (ng/ml)	463.2	402.0	382.3	482.0	342.3	441.3	418.9 $\pm$ 52.8
Y ₁ (ng/ml)	348.6	346.7	356.5	412.5	321.5	395.3	363.5 $\pm$ 33.9
Y ₂ (ng/ml)	110.0	54.2	22.2	68.8	18.4	43.8	52.9 $\pm$ 33.9
C ₀ (ng/ml)	4.61	1.03	3.64	0.71	2.36	2.21	2.43 $\pm$ 1.50
λ_1 (min ⁻¹)	0.1190	0.1020	0.0845	0.0974	0.0793	0.0930	0.0959 $\pm$ 0.0140
λ_2 (min ⁻¹)	0.0231	0.0132	0.0124	0.0142	0.0143	0.0166	0.0156 $\pm$ 0.0039
t _{1/2} λ_2 (min)	30.00	52.35	55.88	48.73	43.41	41.59	43.50 $\pm$ 11.15 ^a
MRT (min)	32.36	45.92	35.41	44.38	29.00	31.36	36.40 $\pm$ 7.09
Cl _B (liter/h·kg)	0.149	0.150	0.196	0.120	0.205	0.168	0.163 $\pm$ 0.0320
Vc (liter/kg)	0.0428	0.0439	0.0533	0.0399	0.0533	0.0410	0.0457 $\pm$ 0.0060
Vss (liter/kg)	0.080	0.115	0.116	0.089	0.099	0.083	0.097 $\pm$ 0.0157
Vd _(area) (liter/kg)	0.179	0.180	0.249	0.135	0.227	0.410	0.23 $\pm$ 0.097
K ₁₀ (min ⁻¹)	0.0596	0.0534	0.0631	0.0531	0.0636	0.0638	0.0594 $\pm$ 0.0050

Y₀, Initial concentration; Y₁ and Y₂ preexponential constants; λ_1 and λ_2 , exponential constants for the biexponential equation describing the plasma concentration time curve; C₀, basal control concentration; t_{1/2} λ_2 , plasma half-time; Cl_B, body clearance; Vss, steady state volume of distribution; Vd_(area) or Vd(β), volume of distribution; K₁₀, first order rate constant of elimination from the central compartment; Vc, volume of the central compartment.

^a Harmonic mean.

TABLE 2B. Individual values and mean ($\pm$ SD) of the kinetic parameters describing oGH disposition in plasma after iv bolus administration of oGH (50 μ g/kg) to the three lambs studied in May

Parameters	G	H	I	Mean $\pm$ SD
Y ₀ (ng/ml)	344.1	263.1	368.7	325.3 $\pm$ 55.3
Y ₁ (ng/ml)	262.6	137.8	337.3	245.9 $\pm$ 100.8
Y ₂ (ng/ml)	77.5	125.2	26.4	76.4 $\pm$ 49.4
C ₀ (ng/ml)	4.00	0.09	4.97	3.02 $\pm$ 2.58
λ_1 (min ⁻¹)	0.0683	0.0457	0.0168	0.0436 $\pm$ 0.0258
λ_2 (min ⁻¹)	0.0151	0.0106	0.0058	0.0105 $\pm$ 0.0047
t _{1/2} λ_2 (min)	45.92	65.67	119.14	66.08 $\pm$ 30.83 ^a
MRT (min)	55.47	76.69	108.86	80.34 $\pm$ 26.88
Cl _B (liter/h·kg)	0.318	0.210	0.106	0.211 $\pm$ 0.106
Vc (liter/kg)	0.147	0.190	0.137	0.158 $\pm$ 0.0282
Vss (liter/kg)	0.294	0.268	0.192	0.251 $\pm$ 0.053
Vd _(area) (liter/kg)	0.645	0.335	0.304	0.428 $\pm$ 0.188
K ₁₀ (min ⁻¹)	0.0379	0.0177	0.0147	0.0234 $\pm$ 0.0126

See Table 2A for definitions.

^a Harmonic mean.

When considering plasma concentrations, no significant difference was found within each state of vigilance or food behavior or according to posture. In contrast, when the instantaneous secretion rates were considered, we observed a significant influence of state of vigilance for $P = 0.072$, food behavior for $P < 0.05$, and posture for $P < 0.02$ (by Friedman test). For each of these three behaviors, the highest production rates were observed dur-

ing rest, i.e. SWS and REM sleep for vigilance, absence of food intake or rumination for feeding, and during lying down for posture.

Discussion

The major finding of these experiments is that GH secretion in sheep is related to behavior (vigilance, feeding behavior, posture). Such a relationship could be evidenced by taking into account the instantaneous GH secretion rate instead of GH concentration profiles.

In man it has been established that the central nervous system plays a major role in the control of GH secretion. A particular relationship between GH secretion and SWS has been demonstrated (1-3). However, in other species, including ruminants (8), such temporal relationships have not been demonstrated. Therefore, the possible role of SWS as a control factor of GH secretion merited investigation. Our results demonstrate the absence of correlation between vigilance, feeding behavior, and posture and plasma GH concentrations. In contrast, the significant relationships existing between such behaviors and instantaneous GH secretion rates lead us to reconsider the negative findings previously reported in different animal species (see *Introduction*).

In this respect, two methodological conditions were required, namely an appropriate sampling schedule and the use of instantaneous secretion rate instead of plasma concentration profiles. A suitable blood-sampling schedule must be selected to fit the frequency of GH pulsatility

TABLE 3A. Mean oGH plasma concentrations and calculated GH production rate in the six lambs studied in January

Parameters	A	B	C	D	E	F	Mean $\pm$ SD
AUC _(0-24 h) (ng/min·ml)	2717.75	11477.85	5726.70	4480.10	3640.70	4593.80	5439.48 $\pm$ 3124.85
Production rate (μ g/kg·24 h)	6.73	28.65	18.70	8.93	12.48	12.13	14.60 $\pm$ 7.99
Mean daily conc. (ng/ml)	1.89	7.97	3.98	3.11	2.53	3.19	3.78 $\pm$ 2.17

TABLE 3B. Mean oGH plasma concentrations and calculated GH production rate in the three lambs studied in May

Parameters	G	H	I	Mean $\pm$ SD
AUC _(0-24 h) (ng·min/ml)	2297.85	3238.20	2588.79	2708.28 $\pm$ 481.43
Production rate (μ g/kg·24 h)	13.88	13.28	4.57	10.57 $\pm$ 5.21
Mean daily conc. (ng/ml)	1.59	2.24	1.80	1.87 $\pm$ 0.33

and the short duration of sleep episodes in animal species. In man a sampling interval of 15 min has been considered optimal for the detection of major episodes of GH secretion (17). Such an interval is too long with respect to the sleep pattern of ruminants, for which the duration of an episode of vigilance is only 5–10 min. Moreover, in studies using polygraphic recording in either rhesus monkeys (3) or goats (8), blood-sampling intervals were too long (15 and 30 min, respectively) to allow the identification of relationships between SWS and GH secretion. Consequently, an intensive sampling procedure allowing GH measurement every 3 min was selected for our investigations.

Nevertheless, whatever the sampling frequency, the direct use of hormonal plasma concentration profile appeared to be of limited interest to demonstrate a correlation between the GH dynamics and any possible controlling events occurring during a short period of time. For instance, in dogs temporal relationships were not found between sleep stage and GH plasma concentrations even with frequent blood samplings (4). This was due to the relatively slow process of GH distribution and clearance, which played the role of a buffer leading to the damping of GH oscillatory concentration.

The solution to the aforementioned problem consisted in taking into account the instantaneous secretion rate instead of plasma concentration. In the experiments reported herein, the secretion rate could be assessed from the plasma concentration profile by deconvolution techniques, which removed the influence of distribution and elimination processes. Currently, several approaches have been suggested. The most general one consists of determining the parameters of hormone disposition from a separate iv experiment; then, knowing both the output function (plasma concentration profiles) and the disposition function, it is possible to solve the input function (instantaneous secretion rate) (19, 27). The main drawback of this analysis arises from the fact that deconvolution is an ill conditioned problem and that noise in data may result in the calculation of negative secretion rates. A solution to this numerical problem is to modelize the input function and solve the convolution integral by considering secretion and clearance parameters as simultaneous unknowns (20). This approach requires some assumptions on the hormone secretion function. In the present experiments, no *a priori* information on input function was available, and we preferred to smooth the data by a spline function, *i.e.* to use a noise-filtering technique to regularize input identification (28).

In our experiments clearance parameters were determined in a separate kinetic study; a bicompartamental model was selected to describe oGH disposition. The clearance was about 0.2 liter/kg·h, a value intermediate between those previously reported in the adult sheep (29, 30). Clearance values were similar for the two groups of lambs, despite the fact that the injected oGH dose was 3 times larger in sheep studied in May. This indicates that the rate of GH elimination was independent of the actual

TABLE 4. Peak characterization by the PULSAR program before [plasma concentration (C)] and after [instantaneous secretion rate (ISR)] deconvolution analysis

	No. of peaks		Amplitude		Peak duration (min)		Total secretory activity (min)	
	C	ISR	C (ng/ml)	ISR (ng/kg·min)	C	ISR	C	ISR
January group (n = 6)	15.67 $\pm$ 4.88	14.66 $\pm$ 4.63	6.62 $\pm$ 3.62	3.18 $\pm$ 1.69	49.93 $\pm$ 15.00	36.83 $\pm$ 9.65	734.00 $\pm$ 122.94	570.00 $\pm$ 318.60
May group (n = 3)	17.33 $\pm$ 4.88	17.66 $\pm$ 4.72	2.50 $\pm$ 0.20	2.86 $\pm$ 0.66	41.83 $\pm$ 5.40	25.00 $\pm$ 2.92	710.00 $\pm$ 72.33	436.00 $\pm$ 84.87
Both groups (n = 9)	16.22 $\pm$ 4.47	15.67 $\pm$ 4.61	5.25 $\pm$ 3.52	3.05 $\pm$ 1.38	47.24 $\pm$ 12.82	32.88 $\pm$ 9.78	726.03 $\pm$ 104.40	525.96 $\pm$ 264.17

Values are the mean $\pm$ SD for either group of lambs and for both groups joined.

FIG. 3. Representative 24-h pattern of GH plasma concentration (*thin line*) and corresponding GH instantaneous secretion rate (SR; *thick line*) in a lamb. Plasma concentrations were measured by RIA in samples obtained every 3 min for 24 h; instantaneous SR were reconstructed by deconvolution analysis in *Materials and Methods*.

TABLE 5. Measured plasma GH concentrations and calculated GH instantaneous secretion rates according to the nine behavioral states investigated

Behavior	Conc. (ng/ml)	Secretion rate (ng/kg·min)
Wakefulness	3.23 ± 2.11	11.21 ± 5.59
Drowsiness	2.79 ± 1.25	12.71 ± 5.75
SWS	2.99 ± 1.64	13.20 ± 5.10
REM	4.31 ± 3.28	17.19 ± 11.32
No food intake	3.23 ± 1.80	12.77 ± 5.86
Food intake	2.91 ± 1.90	9.79 ± 7.06
Rumination	3.33 ± 2.96	11.63 ± 5.43
Standing	3.22 ± 2.20	10.74 ± 4.94
Lying down	3.14 ± 1.95	12.44 ± 5.69

Values are the mean ± SD for the nine lambs under study.

GH concentrations. In contrast, the MRT was significantly longer, and the volume of the central compartment was 3-fold greater in the three sheep dosed in May with 50 µg/kg oGH than in the six sheep dosed in January with 18.5 µg/kg oGH.

The origin of such differences may be either nonlinearity (dose dependence) of GH kinetics, or nonstationarity of GH kinetics with respect to season. It is generally agreed that GH kinetics are linear in both humans (31, 32) and animal species (33, 34), including sheep (29). With respect to clearance, the present results are in agreement with this general opinion. However, a possible dose dependence for the volume of distribution must be expected. Recently, the existence of a specific high affinity, low capacity binding protein for human GH has been reported (35), and beyond a plasma GH concentration of 10 ng/ml, the bound fraction of GH in human plasma is found to vary from about 50% to about 10% of the actual

concentration (36). The presence of such specific proteins in sheep could tentatively explain the dose-dependent increase in the volume of distribution. On the other hand, deconvolution analysis is based on modelling the disposition kinetics of the hormone as a linear system. However, in the present experiments, the nonlinearity of the initial volume of distribution was without effect on estimation of the instantaneous secretion rate. This was due to the fact that the increase in volume of distribution was compensated by a proportionally equivalent decrease in the rate constant of elimination, which allows maintenance of the same clearance regardless of the dose administered.

Another possible origin of variation in the volume of the central compartment could result from a circannual rhythm of GH distribution. In sheep, the photoperiodic control of GH plasma concentration has been demonstrated; GH plasma concentrations increase during the beginning of increasing photoperiod (37). In the present study GH production rates were similar in January and May. It may be observed that a simple decrease in the volume of the central compartment could lead to higher GH concentrations during the increasing day length season, which would suggest an artefactual increase in GH secretion.

The behavioral patterns observed in the present experiments were in close agreement with those previously reported in sheep (21). Based on the instantaneous GH secretion rate, it can be emphasized that regulations of GH secretion, sleep-wakefulness cycle, and feeding behavior are temporally related. However, these relationships remained weak despite their statistical significance. A possible reason could be a lag time between the occurrence of a behavioral event and the GH secretory response. For instance, there is a mean interval of 16 min

between the first SWS epoch and the onset of GH secretion increase in man (38). Another possibility could be the complex interactions among vigilance, feeding behavior, hormonal and metabolic consequences of food intake, and the GH secretion rate. During food intake, sheep are systematically awake, and food intake could be one of the factors explaining the lowest GH values recorded during wakefulness. Indeed, GRF might be involved in feeding behavior of sheep by stimulating eating (39). In addition, according to other researchers, feeding in ruminants may affect the release of somatostatin from the gut and pancreas (40). Therefore, the increases in somatostatin during food ingestion may be able to blunt GRF-induced GH release (39). During sleep, a potentially large increase in GH must be expected due to the absence of food intake. However, rumination occurs mainly during SWS, and most SWS episodes are recorded during rumination (41). One of the purposes of rumination is to favor microbial digestion, and after a period of rumination, an increase in the production of volatile fatty acids (the major energy substrate in ruminants) is observed. Another purpose of rumination is to reduce the residence time of food in the rumen and to aid in maintaining a uniform amount of ingesta in the lower gastrointestinal tract (42). Consequently, rumination with its remastication of food may have some metabolic and/or hormonal consequences similar to those of food intake. This renders the increase in GH secretion rather limited during these periods of SWS/rumination. The decrease in GH secretion rate observed during standing may be the consequence of the relationship among GH secretion, vigilance, and feeding behavior. Indeed, the animals are awake and eat more often when they stand up.

Finally, our results in sheep, a species that has a polyphasic pattern of vigilance with short duration of sleep episodes, are consistent with those reported in man, who exhibits monophasic sleep. The use of instantaneous secretion rate rather than plasma concentrations, which had been considered by other investigators, allowed us to show a relationship between GH secretion and vigilance parameters. It is thus possible that the influence of vigilance, which has only been demonstrated in humans, is, in fact, a general property of other animal species.

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